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The Journal of Allergy

VOL. 18 MARCH, 1947 NO. 2

Original Articles

REACTIONS TO ANTIPNEUMOCOCCAL RABBIT SERUM*†

V. EFFFECTS ON REVERSED PASSIVE ANAPHYLAXIS OF LENGTHENING THE INTERVAL BETWEEN CONSTANT SENSITIZING DOSES OF PNEUMOCOCCAL POLYSACCHARIDE AND CONSTANT SHOCKING DOSES OF ANTIPNEUMOCOCCAL RABBIT SERUM.

VI. DIMINUTION OF CIRCULATING PNEUMOCOCCAL POLYSACCHARIDE.

VII. VARIATIONS IN REVERSED PASSIVE ANAPHYLACTOGENIC PROPERTIES OF DIFFERENT LOTS OF ANTIPNEUMOCOCCAL RABBIT SERUM.

OSCAR SWINEFORD, JR., M.D., MALCOLM A. HOLLIDAY, M.D., PAUL WEBB, M.D., AND H. ROWLAND PEARSALL, M.D., UNIVERSITY, VA.

PROMPT anaphylactic reactions to therapeutic injections of antipneumococcal rabbit serum are common. Conventional anaphylaxis is the accepted mechanism for the reactions which occur when skin tests for sensitivity to rabbit serum are positive. However, tests for sensitivity were negative in nearly 80 per cent of a large series of reactions to antipneumococcal serum.[1] Evidence has been offered that reversed passive anaphylaxis can mediate reactions to antiserum which occur when tests for sensitivity are negative.[2] A series of experiments, now in progress, was started in the hope that some of the problems of reaction to therapeutic antisera would be clarified.

This is the report of: (1) the effect on reversed passive anaphylaxis of increasing the interval between a constant sensitizing dose of polysaccharide and a constant shocking dose of antiserum, (2) some observations on the diminution of circulating polysaccharide after intravenous injection, and (3) the apparent differences in reversed passive anaphylactogenic properties of several lots of antiserum.

Experiment V. Effect of increasing interval between constant sensitizing dose of polysaccharide and constant shocking dose of antiserum.—Guinea pigs weighing 400 to 875 Gm. were given constant sensitizing doses of 1.5 mg. of type I pneumococcal polysaccharide intravenously At lengthening intervals,

*From the Allergy Clinic, Department of Internal Medicine, University of Virginia Medical School, Charlottesville, Va.

†This is one of several studies made possible by a grant plus generous supplies of antipneumococcal rabbit serum by Wyeth, Inc.

up to ten days, they were given shocking doses of 2.0 ml. (14,000 units, lots No. 338 and A.338.2) of type I antipneumococcal rabbit serum intravenously. The sensitizing and shocking doses had been shown[3] to be effective in producing reversed passive anaphylaxis quite regularly when the interval was about one hour. The polysaccharide[1, 3] had been shown to be nontoxic for guinea pigs. As negative controls two guinea pigs were given 2.0 ml. of lot No. 338 and three were given 2.0 ml. of lot No. A.338.2 of antiserum. Four other pigs were given 1.5 mg. type II polysaccharide, and then 2.0 ml. of lot No. A.338.2 type I antiserum one hour later. There were no reactions. The negative reactions in Tables I and II serve as additional negative controls. Further details of the experiment are shown in Table I. It can be seen that reversed passive anaphylaxis occurred regularly for seven days after the sensitizing injection of polysaccharide. There was one slight reaction on the eighth day but none on the ninth or tenth days.

TABLE I. EFFECTS OF LENGTHENING THE INTERVAL BETWEEN SENSITIZING DOSE OF PNEUMOCOCCAL POLYSACCHARIDE AND SHOCKING DOSE OF ANTIPNEUMOCOCCAL RABBIT SERUM ON REVERSED PASSIVE ANAPHYLAXIS IN GUINEA PIGS

Sensitizing Dose = 1.5 mg. type I pneumococcal polysaccharide.
Shocking Dose = 2.0 ml. type I antipneumococcal rabbit serum Lots No. 338 and A.338.2, 7,000 units/ml.

INTERVAL (DAYS)	REACTION*				CONTROLS
1	AD	AD	++	AD	See Text
2	AD†	AD			
3	++†	AD	++†		
4	+†	AD			
5	AD	AD			
6	AD	AD	AD		
7	++	AD	AD		
8	+	0	0†	0	
9	0	0			
10	0	0	0	0	

*0 = No reaction.
+ = Slight anaphylactic reaction = ruffled hair, sick, expiratory dyspnea.
++ = Moderate to marked reaction = quite sick, convulsive movements, marked dyspnea.
AD = Anaphylactic death, necropsy = pale, ballooned lungs, scattered hemorrhagic areas, bronchial exudate.
†Animal struggled, less than 2.0 ml. antiserum given.

Experiment VI. Diminution of circulating pneumococcal polysaccharide.—Titration of persisting circulating polysaccharide was suggested by the regular production of reversed passive anaphylaxis for seven days after the injection of antigen. Two groups of guinea pigs were studied.

Group A, 30 animals, varied in weight from 315 to 560 Gm. Each animal was given 1.0 mg. of type I pneumococcal polysaccharide intravenously. Serum was obtained by venesection from two of the 30 animals daily for fourteen days and from two others on the nineteenth day. Persisting polysaccharide was precipitated in a capillary "J" tube[3] from 0.1 ml. guinea-pig serum by 0.5 antipneumococcal rabbit serum, lot No. 409.1, 7,000 units/ml. The volumes of precipitate in cu. ml. were averaged for each day and were charted as A in Fig. 1. One titration on the second day is missing.

Group B, 31 animals, varied from 400 to 875 Gm. Each animal was given 1.5 mg. type I pneumococcal polysaccharide intravenously. Serum was obtained

from several animals daily for twelve days. Polysaccharide was precipitated in capillary "J" tubes from 0.1 ml. guinea-pig serum by 0.04 ml. (500 units) of antipneumococcal rabbit serum lot No. A.338.2, 7,000 units/ml. The volumes of precipitate were averaged for each day and were charted as B in Fig. 1. Both curves showed progressive but irregular diminution of polysaccharide although they have wide numerical differences. Other titrations not recorded show the same trend but still different numerical values for persisting circulating polysaccharide. Controls are shown in the figure.

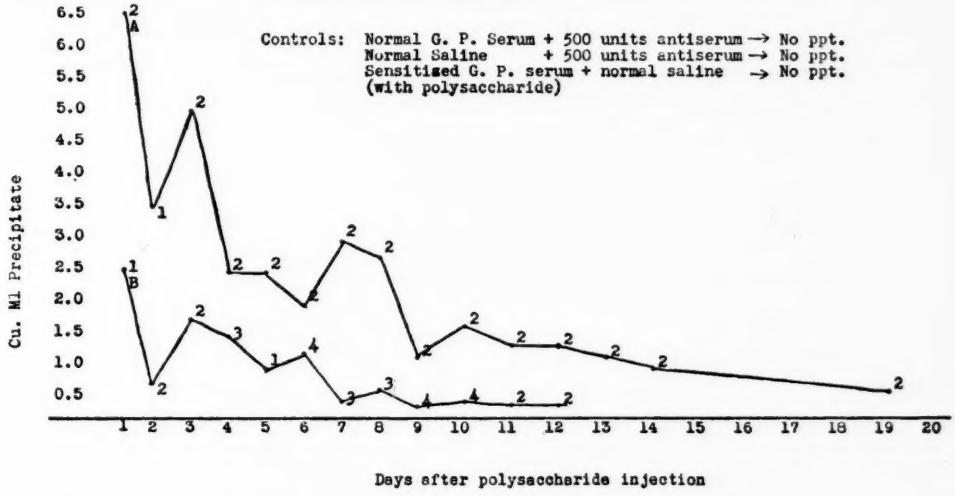
Controls: Normal G. P. Serum + 500 units antiserum → No ppt.
Normal Saline + 500 units antiserum → No ppt.
Sensitized G. P. serum + normal saline → No ppt.
(with polysaccharide)
Cu. Ml Precipitate
Days after polysaccharide injection

Fig. 1.—Diminution of circulating pneumococcal polysaccharide.

A.—1.0 mg. type I polysaccharide intravenously into guinea pigs. Polysaccharide precipitated from 0.1 ml. guinea pig serum by 0.5 ml. of lot No. 409.1 antipneumococcal rabbit serum.

B.—1.5 mg. type I polysaccharide intravenously into guinea pigs. Polysaccharide precipitated from 0.1 ml. guinea pig serum by 0.04 ml. of lot No. A.338.2 antipneumococcal rabbit serum.

Numerals indicate number of precipitate volumes averaged for each point.
Each precipitate from serum of different animal.

Experiment VII. Differences in reversed passive anaphylactogenic properties of several lots of type I antipneumococcal rabbit serum.—In earlier experiments, not reported, several lots of antiserum seemed less capable of producing reversed passive anaphylaxis than others. Comparisons of the effectiveness of four lots of antiserum in causing shock in animals with diminishing circulating polysaccharide are presented in Table II. It is apparent that serum lots No. 338 and A.338.2 (7,000 units/ml.) had comparable effects, causing marked reversed passive anaphylaxis up to seven days after the sensitizing injections of polysaccharide. In sharp contrast are the effects of lot No. 401.02 (4,000 units/ml.) which caused only one anaphylactic death and no reaction after the fourth day and lot No. 305.1 (7,000 units/ml.) which caused only one slight reaction after the second day.

DISCUSSION

Experiment V. Reversed passive anaphylaxis in guinea pigs has occurred, so far, only when there is an excess of circulating antigen, when the sensitizing dose of polysaccharide is injected one hour before the shocking dose of

TABLE II. COMPARISON OF REVERSED ANAPHYLACTOGENIC PROPERTIES OF FOUR DIFFERENT LOTS OF TYPE I ANTIPNEUMOCOCCAL RABBIT SERUM

Sensitizing Dose = 1.5 mg. type I pneumococcal polysaccharide (except 1.0 mg. used in No. 305.1 and some of A.338.2 columns).
Shocking Dose = 2.0 ml. type I antipneumococcal rabbit serum.

INTERVAL	LOT NO. 338 7,000 UNITS/ML.	REACTION† LOT NO. A.338.2 7,000 UNITS/ML.	LOT NO. 401.02 4,000 UNITS/ML.	LOT NO. 305.1 7,000 UNITS/ML.
1 hour		AD AD	0 ± 0	AD AD
1 day	AD AD ++ AD	AD	++ AD	0 AD
2 days	AD† AD	AD	+ ++	AD AD
3 days	++ AD ++†	AD AD	+ 0	+ 0
4 days	+† AD	++ + AD	++	0 0
5 days	AD AD	+ + AD		
6 days	AD	AD 0 AD AD	0	
7 days	++ AD	AD ++ + 0		
8 days	+ 0	0 0 0	0	
9 days		0 0 0 0		
10 days		0 0 0 0 0	0	
11 days		0	0	
12 days		0	0	

*0, +, ++, and AD = same as Table I.
†Animal struggled, less than 2.0 ml. antiserum given.

antiserum.[3] This experiment (Table I) supports the conclusion that an excess of circulating polysaccharide is necessary to the production of reversed passive anaphylaxis. The difference here is that constant and considerable excesses of sensitizing polysaccharide, 1.5 mg., were injected and constant shocking doses of 2.0 ml., 14,000 units, of antiserum were given at lengthening intervals thereafter. Reversed passive anaphylaxis occurred regularly for seven days, presumably as long as there was sufficient circulating antigen. No effort was made to study shorter intervals than one hour.

Experiment VI (Fig. 1) shows that circulating polysaccharide persists longer than seven days in amounts detected easily in a capillary "J" tube. Note that smaller amounts of polysaccharide and larger amounts of antiserum were used in A than in B. The quantitative differences between A and B and another shorter curve have not been explained. None of the data obtained in these or in preliminary experiments, not detailed, permit an estimate of the minimal amount of polysaccharide in mg./ml. serum necessary for reversed passive anaphylaxis. Before such an estimate can be made it will be necessary to evaluate the role of the weight and blood-volumes of guinea pigs, the optimal amount of precipitating antiserum, the optimal shock dose of antiserum, and the excretion and metabolism of polysaccharide.

There is a clinical analogue of this persistence of circulating polysaccharide, with its potentialities for reversed passive anaphylaxis upon administration of antiserum. In cases of pneumonia with poor antibody-response, Bullowa and others[4] have demonstrated the persistence of circulating polysaccharide. It is conceivable, too, that an excess of polysaccharide could occur in those complications of pneumonia in which active foci of pneumococci persist. By analogy, then, it would seem proper to determine the presence of circulating polysaccharide before giving therapeutic antipneumococcal serum. By analogy also, if definite amounts of circulating polysaccharide are found, antiserum should be

given only after consideration of the possibilities of reversed passive anaphylaxis.

These observations should not be applied too literally, yet, to the therapeutic use of antiserum which has saved the lives of many extremely ill patients, without untoward reactions.

The persistence of easily detectable amounts of circulating polysaccharide nineteen days after the injection in the two animals studied was a striking finding.

Experiment VII (Table II) shows that there are variations in anaphylactogenic properties of different lots of antipneumococcal rabbit serum. In other experiments, not detailed, 8,000 units is in excess of the amount of potent antiserum required for an effective shocking dose. This variation in antisera is another obstacle to the estimation of the minimal amount of polysaccharide necessary to the production of reversed passive anaphylaxis in guinea pigs. Isolated attempts[2, 5] to separate the anaphylactic antibody from precipitins have failed. The apparent differences in these sera suggest the need to investigate the propriety of testing batches of serum not only for primary toxicity[2] but also for abnormally high anaphylactogenic potency.

SUMMARY

Additional studies of reactions to antipneumococcal rabbit serum are presented which show:

1. That reversed passive anaphylaxis can be produced in guinea pigs by a constant shocking dose of potent antiserum as long as seven days after a constant sensitizing dose of an excess of specific pneumococcal polysaccharide.

2. That single intravenous injections of pneumococcal polysaccharide can be detected easily, in diminishing amounts in 0.1 ml. of guinea-pig serum, for periods up to nineteen days (perhaps longer).

3. That different lots of antipneumococcal rabbit serum seem to vary in their anaphylactogenic properties.

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DELAYED ABSORPTION OF PROTEINS IN THE GUINEA PIG*

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IN THESE experiments an effort has been made to determine the factors that may influence the absorption of foreign protein after subcutaneous injection.

It has been shown that on subcutaneous injection inert particles of certain molecular weights proceed from the tissues by way of the lymphatics before reaching the blood stream, while other particles of smaller molecular weight will be absorbed directly into the blood stream. The exact size of molecule that will enter a blood capillary is not known, but the approximate maximal size of such molecules has been determined. Molecules of larger size will travel by the lymphatic vessels after subcutaneous injection. Lewis,[1] and later Field and Drinker[2] have shown that serum proteins are absorbed from the tissues and are carried to the blood by the lymphatics. Their molecular weight is in the neighborhood of 67,100.[3] Barnes and Trueta[4] carried out experiments with diphtheria toxin, which was shown to have a molecular weight of 70,000,[5] and found that the absorption from subcutaneous sites was considerably delayed by immobilizing the limb in a plaster spica, indicating that this toxin travels through the lymphatics. These authors also tested cobra venom, whose molecular weight is in the neighborhood of 3,000, and found that it was readily absorbed directly into the blood stream after subcutaneous injection and exerted more immediate effects.

Abramson, Moore, and Gettner[6] calculated the weight of the active molecules in ragweed extract by the ultracentrifugal method to be about 5,000. Sanigar[7] found the molecular size in unpurified ragweed extracts to be heterogeneous, but the majority were less than 17,000. The smallest molecular weights of true proteins, for example, lactalbumin, approximate 17,600.[3] Saline suspensions of the active molecule of such substances of low molecular weight on subcutaneous injection will probably be absorbed directly into the blood capillaries rather than through the lymphatic channels.

It has also been shown that the molecular weight as determined chemically is not the sole determining factor, but also the state of the molecules as they exist in solution, has some influence on the rapidity of absorption of individual particles. Certain substances, such as toxins and serum proteins, have a fairly uniform molecular size and shape while others, such as the dye trypan blue[8] and the synthetic colloids[9] show considerable variation in size and shape which tends to delay their absorption after injection. These synthetic colloids have filamentary molecules, not globular ones like the plasma albumins and globulins.

In these experiments two methods have been used to delay the absorption of subcutaneously administered foreign protein. One method was the use of

*From the Allergy Clinic, Hospital for Sick Children and the Department of Pediatrics, University of Toronto, under the direction of Alan Brown, M.D.

colloidal solutions and the other was the method of precipitation of the protein molecules using coagulating substances.

A preliminary series of experiments was done to determine the effectiveness of ragweed antigen in shocking sensitized guinea pigs. In these experiments it was found that ragweed was not a suitable antigen to use as the animals could not uniformly be fatally shocked, even when alum-precipitated pollen extracts were used.[10] Purified egg albumin was then used as an antigen in all subsequent experiments and this was found to give uniform and predictable results in practically every instance.

The purpose of the first experiment was to determine the minimal amount of purified egg albumin* required to produce fatal anaphylactic shock in sensitized guinea pigs by subcutaneous injection. Groups of guinea pigs of standard breed weighing approximately 200 Gm. were used. These were fed a standard diet of rabbit chow and greens. The animals were divided into groups with equal numbers of males and females in each group. They were then sensitized by a single intra-abdominal injection of 1 ml. of 5 per cent egg albumin in saline. After twenty-eight days the pigs weighed approximately 350 to 400 Gm., and appeared perfectly healthy. Animals in Group I were then injected subcutaneously with 1 ml. of 5 per cent aqueous egg albumin in varying dilutions and the symptoms of anaphylactic shock observed. Symptoms of shock were recorded according to the classification of Wells and Osborne.[11] In many instances rectal temperatures were also recorded before and at intervals after the shock dose was given. It was found that the minor symptoms of shock were variable and somewhat unreliable in these animals, and for this reason fatal anaphylaxis was the only criterion on which the results are based. Fatal anaphylaxis occurred at varying time intervals according to the dose administered. With dosages approaching the critical dose the animal will die in from two to eight hours. With larger dosages death occurs in one-half to two hours.

From Chart I it is seen that the critical dose of purified egg albumin in aqueous solution required for fatal shock is 12.5 mg.

Experiment 2 was done to determine the effect of injecting suspensions of egg albumin in polyvinyl alcohol. After preliminary experiments using various colloids such as gelatin, gum acacia, and agar, polyvinyl alcohol (P.V.A.) type Rh 623† was found to be the most suitable for our purpose. Roome, Ruttle, Williams, and Smith[12] have used the polyvinyl alcohols intravenously in shock and other cases and find that they are well tolerated, showing no evidence of foreign body reaction or other injurious effects after repeated injections. The polyvinyl alcohols are a series of water-soluble colloids produced commercially in a number of grades. Each grade is a mixture of various sized macromolecules, the product of polymerization to various degrees, and the properties vary with the average molecular size. They are white powders with very little odor or taste and are stable on heating or boiling in aqueous solutions. Solutions of 5 per cent or over form stable gels on standing at ordinary

*Albumin Egg, Merck, soluble.
†Kindly supplied by Canadian Industries Limited (Canada).

ANTIGEN INJECTED MG.	CHART I SHOCKED WITH EGG ALBUMIN IN AQUEOUS SOLUTION	CHART II SHOCKED WITH EGG ALBUMIN IN POLYVINYL ALCOHOL	CHART III SHOCKED WITH EGG ALBUMIN TREATED WITH PROTAMINE ZINC AND SUSPENDED IN POLYVINYL ALCOHOL
55			• 2 pigs died. 0 survived
			• 2 pigs died. 0 survived
50			*Critical Dose* ⊙ 4 pigs survived. 0 died
			• 3 pigs survived. 1 died
45			• 3 pigs survived. 0 died
40			• 1 pig survived. 0 died
35			• 1 pig survived. 0 died
		• 3 pigs died. 0 survived	
30		• 4 pigs died. 0 survived	• 1 pig survived. 0 died
		Critical Dose ⊙ 3 pigs survived. 1 died	• 1 pig survived. 0 died
25		• 3 pigs survived. 1 died	
		• 4 pigs survived. 0 died	
20		• 2 pigs survived. 0 died	
	• 2 pigs died. 0 survived	• 1 pig survived. 0 died	
15	• 5 pigs died. 1 survived	• 1 pig survived. 0 died	
	Critical Dose ⊙ 6 pigs survived. 0 died	• 1 pig survived. 0 died	
10	• 6 pigs survived. 1 died		
	• 4 pigs survived. 0 died		
5	• 2 pigs survived. 0 died		

Charts I, II, and III.—Guinea pigs shocked with varying dilutions of purified egg albumin.

temperatures. The powder is best dissolved by mixing with a small quantity of chilled water, warming, and finally adding water to full volume; any salts to be added should be dissolved in the second portion of water. Such solutions may contain small numbers of microscopic particles and may be passed through a Seitz filter if they are to be used for subcutaneous injection. Solutions may be sterilized by autoclaving or boiling.

A series of dilutions of purified egg albumin in 10 per cent polyvinyl alcohol were injected subcutaneously into sensitized guinea pigs at the end of twenty-eight days. A uniform volume of 1 ml. was injected in each instance, and the animals were observed for evidence of shock. From Chart II it is seen that the critical dosage is 27.5 mg. of purified egg albumin in 10 per cent polyvinyl alcohol.

Experiment 3 was done to determine the effect of protamine zinc on the absorption of egg albumin suspensions in polyvinyl alcohol.

A series of dilutions of purified egg albumin were set up and 1 mg. ZnO was added to each. Then fresh solutions of protamine* were prepared and adjusted to pH 2.5, and 0.6 mg. were added to each tube. The reaction was adjusted to neutral with phosphate buffer, which results in a fine precipitate. These solutions were then suspended in polyvinyl alcohol to a final concentration of 10 per cent and 1 ml. injected subcutaneously into each sensitized guinea pig. The results of this experiment (Chart III) show that the critical dose of protamine-zinc-treated egg albumin when suspended in polyvinyl alcohol is 50.0 mg.

SUMMARY

1. From these experiments it is seen that when untreated solutions of purified egg albumin are injected subcutaneously into sensitized guinea pigs, fatal anaphylaxis is produced if the dosage is in excess of 12.5 mg.

2. If similar solutions are suspended in 10 per cent polyvinyl alcohol, fatal anaphylaxis is not produced until 27.5 mg. of egg albumin are injected.

3. If similar solutions are treated with protamine-zinc and then suspended in 10 per cent polyvinyl alcohol, fatal anaphylaxis is not produced until 50.0 mg. are injected.

4. This delay in the production of fatal anaphylactic shock has probably been effected by a delay in the absorption of the antigen from the subcutaneous tissues, and in experiment 3 it is seen that four times the dose of antigen is tolerated by the pigs. The delayed reaction probably results from the slower absorption of the antigen through lymph channels rather than the more direct absorption through the capillaries.

5. Fatal anaphylaxis is only produced in the guinea pig when amounts of antigen in excess of 12.5 mg. are absorbed during a short period, probably only a few minutes. If the absorption is delayed sufficiently, so that amounts less than 12.5 mg. are absorbed at any one moment, but are absorbed continuously over a period of hours, the animal will not suffer fatal shock.

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*Extract of mature salmon testes.

QUANTITATIVE CHANGES IN VARIOUS FRACTIONS OF THE PRECIPITABLE NITROGEN IN RAGWEED EXTRACTS DURING INCUBATION (37° C.)*†

FRANKLIN A. STEVENS, M.D., NEW YORK, N. Y.

THE deterioration of pollen extracts on ageing is commonly recognized. In 1933 Stull, Cooke, and Tennant[1] observed a diminution in phosphotungstic acid precipitable nitrogen[2] in aged extracts. Later, Stull, Sherman and Wing[3] analyzed a low ragweed extract before and after incubation. They observed a loss of "protein nitrogen" (phosphotungstic acid precipitable) of a Fraction 1 ("protein nitrogen" precipitated by half saturation with ammonium sulfate) and of a Fraction 2 (containing "protein nitrogen" precipitated by 50 to 100 per cent saturation with ammonium sulfate). Nitrogen of a Fraction 3 (precipitated by acidification to pH 4) did not diminish quantitatively during incubation. Both Fractions 1 and 2 have been isolated from extracts of low and giant ragweed.[4]

In the following experiments determinations have been made of the nitrogen precipitated by half and full saturation with sodium sulfate and by phosphotungstic acid at various intervals during the incubation of a giant ragweed extract.

EXPERIMENTAL

A single lot of giant ragweed pollen defatted by ethyl ether (1944) was used to make the extracts in the following experiments. For each, 20 Gm. of pollen were extracted by repeated washings with distilled water at 4° C. These washings were then combined and made up to approximately 200 c.c. After centrifugation at 4° C., the extract was adjusted to the pH required with N/10- or N/100 HCl (Table I) and filtered cold by suction through hardened filtered paper. The volumes of extract in different experiments were not constant. The extract was then saturated with toluene and centrifuged cold to remove the excess. For each experiment in a number of stoppered volumetric tubes were prepared so that duplicate tubes would be available for each contemplated analysis. The extract was warmed quickly to 37° C., and 10 c.c. were pipetted into each tube. Eight of these tubes were analyzed immediately. After a drop of toluene had been added to each tube, the remaining tubes were incubated at 37° C. for future analysis. Each tube was cultured before analysis. An occasional tube was found contaminated and discarded. Total nitrogen, and the nitrogen precipitated by half and full saturation with sodium sulfate and by phosphotungstic acid were determined. The total nitrogen was done directly by micro-Kjeldahl. The remaining calculations were by difference. Those for nitrogen at half saturation were carried out by adding an equal volume of a solution of anhydrous sodium sulfate saturated at 37° C. (44.4 Gm. per cent). After adjusting to pH 4 with diluted HCl and adjusting the volume, the extract was

*From the Department of Medicine, Columbia University College of Physicians and Surgeons, and the Presbyterian Hospital.

†The pollen used in these experiments was supplied by Wyeth, Inc.

TABLE I. QUANTITATIVE CHANGES IN THE PRECIPITABLE NITROGEN IN RAGWEED EXTRACTS

EXPT.	pH OF EXTRACT	DAY OF ANALYSIS	MAINTAINED T. OF EXTRACTS DEGREES C.	TOTAL N MG.	NITROGEN PRECIPITATED BY		
					HALF SATURATION WITH Na_2SO_4 MG.	FULL SATURATION WITH Na_2SO_4 MG.	PHOSPHOTUNGSTIC ACID MG.
1	6.0	0	4	6.78	.78		3.58
		6	37		.18	.82	3.16
		11	37		.09	.84	3.13
		19	37				3.10
2	6.9	0	4	6.90	.95	1.87	3.38
		7	37		.12	.98	3.30
		19	37		.08	.31	2.90
3	6.8	0	4	7.08	.92	1.12†	3.53
		6	37		.28	.74†	2.98
4*	6.8	0	4	7.08	.92	1.12†	3.53
		7	4		.91	1.14†	3.45
5	7.4	0	4	6.73	1.21	1.65	3.21
		7	37		.26	1.13	2.87
6*	7.4	0	4	6.73	1.21	1.65	3.21
		14	4		1.10	1.50	3.08

*Portions of Extracts 3 and 5 kept at 4° C. for 7 and 14 days, respectively, were warmed rapidly at 37° C., pipetted at this temperature and analyzed immediately.

†N precipitated by three quarters instead of full saturation.

filtered. After flocculation had occurred, the precipitate was washed with half saturated sodium sulfate, and the nitrogen in the filtrate determined. The total nitrogen in the filtrate was subtracted from the total nitrogen in 10 c.c. of extract. All volumes were at 37° C. The full saturations with sodium sulfate were done in the same manner, except anhydrous sodium sulfate was added to 44.4 Gm. per cent at 37° C. The method for phosphotungstic acid precipitable N has been described in the literature (Stull et al.[2]).

DISCUSSION

The data submitted show a decrease in the nitrogen precipitated at both half and full saturation during incubation at 37° C. The nitrogen precipitated by phosphotungstic acid also shows a decrease, but the loss is proportionately less. While the figures cannot indicate absolutely the quantities of protein precipitated by the reagents because some nonprotein nitrogen and products of protein disintegration must adhere to the precipitates, the analyses sugest a degradation of protein molecules to split products of lesser and lesser magnitudes to a point where they are beyond the effective level of the precipitants employed. This conversion occurs rapidly at 37° C. and slowly at refrigerating temperatures. In discussing an experiment in which three "protein" fractions were determined quantitatively by difference through the use of various precipitants in extracts of ragweed before and after incubation, Stull, Sherman, and Wing[3] admit the possibility that split protein products in fresh and incubated extracts accumulate and may be precipitated by phosphotungstic acid. The present study affords experimental evidence supporting this view.

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SENSITIZATION BY TOPICAL APPLICATION OF SULFONAMIDES*

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SINCE the first introduction of the sulfonamide drugs, they have been employed topically for the treatment of skin ailments. It was quickly realized, however, that sensitization may follow repeated skin application. This awareness reached its height in an official Army Medical Bulletin[1] which stressed the urgency of limiting topical applications to only five or six days.

Despite the knowledge that local application of sulfonamides may produce undesirable side-effects, there is little exact information about the relative incidence of sensitization by the different members of the sulfonamide series, the factors influencing the incidence of sensitization, the effect of previous systemic exposure to the drug on the incidence of sensitization following external applications, the value of the patch test in demonstrating the skin's hypersensitivity, the effect of subsequent systemic administration of the drug to those sensitized by external applications, the incidence and scope of cross sensitizations, and other matters of theoretical and practical interest. The present report is an attempt to answer some of these questions.

In the course of studies on the treatment of third degree burns in man, we made several hundred standard lesions to which we applied measured amounts of four different sulfonamide creams.

The burns were produced by the Henriquez-Moritz burn apparatus (Harvard University). This apparatus consists of a copper disc 1.0 cm. in diameter, heated to a temperature of 67° C. The disc forms the bottom of a container in which methanol is boiled by passage of an electric current through a high-resistance wire in the wall of the container. The heated disc is applied to the skin of each forearm for sixty seconds. No pressure is used except that exerted by the weight of the applicator itself. Uniform, symmetrically placed lesions were thus produced on the volar aspects of the forearms of each volunteer. Histologic examinations showed that the lesion was a third degree burn with very rare small rests of epithelial cells, mostly the deeper portions of the skin glands and follicles. Acid débridement was used to remove the slough, as elsewhere described.[2] As soon as débridement was complete, the treatment was changed to daily dressings with a sulfonamide-containing preparation, either sodium sulfadiazine cream, sulfadiazine cream, sulfathiazole cream, or sulfanilamide cream. About 8.0 c.c. of the cream was applied daily to each of the two lesions. The preparation in each instance consisted of 5 per cent of the sulfonamide in a standard water-miscible base:

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Sodium benzoate, U.S.P.	0.2%
Stearic acid, U.S.P.	10.0
Glyceryl stearate	5.0
Glyceryl laurate	5.0
Glycerin	10.0
Distilled water g.s.ad	100.0

The 5 per cent sodium sulfadiazine cream was supplied by the U. S. Navy and was the formula in general use in the field. The sulfadiazine, sulfanilamide, and sulfathiazole creams were prepared especially* for this study, using the same concentration in the same vehicle. Many of the men volunteered a second and a third time. When they did, the same sulfonamide was applied for all courses of treatment. This enabled us to make repeated series of applications of the same sulfonamide for successive burns in some cases.

INCIDENCE OF SULFONAMIDE DERMATITIS FOLLOWING TOPICAL TREATMENT OF BURNS WITH SULFONAMIDE CREAM

There is a paucity of exact statements concerning the incidence of sensitization following topical application. Many large series have been reported in which local treatment with sulfathiazole ointment was employed without the development of sensitization.[3-7] Cohen[8] reported a series of 69 cases of impetigo treated with sulfanilamide cream with no obvious sensitization. Some authors have reported instances of sensitization after treatment with sulfathiazole, usually confirmed by reaction to subsequent oral administration rather than by patch tests.[9-12] Livingood and Pillsbury[13] did attempt an estimate of incidence. They reported 12 instances of dermatitis in a total of "over 1,000 men" treated with sulfathiazole. Darke[14] found 12 cases of dermatitis in 218 patients treated with sulfathiazole, an incidence of 5.5 per cent.

Tate and Klorfajn[15] reported 55 cases of sulfonamide dermatitis among 2,289 admissions to the skin department of a military hospital. Many of these were examples of sulfanilamide sensitivity.

In our study, we had an opportunity to observe the incidence of dermatitis following treatment with *measured amounts* of different sulfonamides in the *same vehicle,* on *standard lesions,* in men of the *same age group, living in the same environment, on the same diets, at the same occupation, and under similar experimental conditions.*

Table I shows that the incidence of dermatitis for all four sulfonamides in a total of 254 men was 19 per cent. However, there was a considerable variation as to the incidence of dermatitis among the four groups. Treatment with the sodium sulfadiazine resulted in a dermatitis in over one-half of the men, while treatment with sulfanilamide, the agent chiefly reported as used in the British Army, caused dermatitis in almost one-fourth of the men. Treatment with sulfathiazole, the agent most frequently used in civilian life, caused dermatitis in 7 per cent of the men, and similar application of sulfadiazine, used by the U. S. Army, gave the lowest incidence of sensitization, 5 per cent. It

*We are indebted to the Wallace Laboratories, New Brunswick, N. J., for preparing and supplying these materials.

TABLE I. INCIDENCE OF SENSITIZATION AFTER TREATMENT WITH VARIOUS SULFONAMIDES

DEGREE OF SENSITIZATION	SODIUM SULFADIAZINE 49 SUBJECTS		SULFANILAMIDE 54 SUBJECTS		SULFATHIAZOLE 72 SUBJECTS		SULFADIAZINE 79 SUBJECTS		TOTAL 254 SUBJECTS	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Severe bilateral dermatitis	5	10.0	5	9.0	0	0.0	0	0.0	10	4.0
Moderate dermatitis	2	4.0	5	9.0	1	1.4	0	0.0	8	3.0
Slight bilateral dermatitis	14	28.0	0	0.0	1	1.4	1	1.3	16	7.0
Slight unilateral dermatitis	7	14.0	2	4.0	3	4.0	3	4.0	15	6.0
Total incidence of dermatitis	28	57.0	12	22.0	5	7.0	4	5.0	49	19.3
Positive patch tests; no dermatitis	2	4.0	0	0.0	1	1.4	0	0.0	3	1.0
Clinical dermatitis plus positive patch reactions	30	61.0	12	22.0	6	8.4	4	5.0	52	20.5

TABLE II. NUMBER OF APPLICATIONS AND DAYS OF EXPOSURE

SULFONAMIDE TREATMENT; SERIES AND NUMBER OF SUBJECTS	SODIUM SULFADIAZINE 49 SUBJECTS	SULFANILAMIDE 54 SUBJECTS	SULFATHIAZOLE 72 SUBJECTS	SULFADIAZINE 79 SUBJECTS
Average number of applications for the series	25.6	24.7	20.3	19.9
Average number of days of exposure for the series	15.4	13.7	11.9	11.5

will be recognized that the relative *incidence of sensitization corresponded to the relative water-solubility* of the compounds—the most soluble giving the highest incidence of sensitization.

Table II shows the average number of applications and of days each ointment was applied. When the cream was applied to both arms on one day, this was counted as two applications. Since one forearm often healed more quickly than the other (for the reason that the burns on the two arms had different types of preceding treatment), one arm often received the sulfonamide before the other and the average *number of applications* bears no constant relation to the *number of days* applied. Study of Table II reveals that the sodium sulfadiazine series and the sulfanilamide series were closely similar, both as to number of men treated and the number of applications and days of treatment. Nevertheless, the incidence of dermatitis in the sodium sulfadiazine group was two and one-half times greater than in the sulfanilamide group. The two series treated with sulfathiazole and sulfadiazine are similarly comparable in quantity and time of exposures, and in these two series the incidence of dermatitis was about equal. The average number of applications per man in the first two groups was only five more than for the second two groups. It appears that the difference in incidence of dermatitis between the first two groups and the second two groups is much greater than could be explained by the difference in number of applications. Moreover, by referring to Table I, it will be seen that the *intensity* of dermatitis was greater in the first two than in the last two groups. These results indicate that the first two drugs (sodium sulfadiazine

and sulfanilamide) have a greater sensitizing potential than the second two (sulfathiazole and sulfadiazine). In the sodium sulfadiazine series it seems that there is a direct relationship between the number of applications and the incidence of dermatitis (Table III).* This relationship is not seen in the other series. The point is of importance because several authors have stressed the desirability of limiting in each case the number of topical applications to a certain maximum, rather than the need for eliminating the topical application of the drugs.[1, 16]

TABLE III. RELATION OF AMOUNT AND DURATION OF EXPOSURE TO INCIDENCE OF DERMATITIS

	SODIUM SULFADIAZINE		SULFANILAMIDE		SULFATHIAZOLE		SULFADIAZINE	
	WITH DERMATITIS	NO DERMATITIS	WITH DERMATITIS	NO DERMATITIS	WITH DERMATITIS	NO DERMATITIS	WITH DERMATITIS	NO DERMATITIS
Average number of applications	34.0	15.7	16.2	23.6	26.2	21.4	18.2	20.2
Average number of days of exposure	20.0	9.8	10.5	13.0	14.5	12.4	10.0	12.6

TABLE IV. INFLUENCE OF PREVIOUS (ACID) DERMATITIS OF THE SITE ON THE INCIDENCE OF SULFONAMIDE SENSITIZATION

TREATMENT SULFONAMIDE	INDEX OF PREVIOUS (ACID) DERMATITIS*	
	SENSITIZED SUBJECTS	UNSENSITIZED SUBJECTS
Sodium Sulfadiazine	2.40	2.10
Sulfanilamide	0.92	0.84
Sulfathiazole	2.80	1.62
Sulfadiazine	3.00	1.77
Total all cases	2.12	1.58

*Index determined as follows: Each case of slight acid dermatitis was given a score of 1.0; of moderate dermatitis a score of 2.0; of severe dermatitis a score of 3.0. The figures were totaled for all the arms in each group and divided by the number of men in the group. The resulting number is the "index" of the degree of (acid) dermatitis existing before application of the sulfonamide cream.

The prior application of the various acids to produce débridement caused different degrees of irritation and injury to be present on the areas to which the sulfonamide creams were subsequently applied. Since several authors have stressed the fact that previous skin injury seemed to predispose to development of sulfonamide dermatitis,[12, 13, 15, 17]† we noted carefully the degree of acid injury in each case. In some men the acid produced only different degrees of redness. In others, there was also edema, vesiculation, and scaling. In the most severe cases there were, in addition, numerous small necrotic spots which later developed into ulcers. An index of previous injury caused by the application of the acid was estimated in each series by the method described in Table IV. The figures of incidence of sensitization shown in this table tend to

*Of course the *area* of application was always approximately double the size when both arms were treated as when only one arm received treatment. This, too, may have had some influence on the incidence of sensitization in the sodium sulfadiazine series.

†Sulzberger and Wise first demonstrated this type of increased incidence of sensitization on topical application of butesin picrate ointment to injured skin (Drug Eruptions II. Dermatitis Eczematosa Due to Drugs, Arch. Dermat. & Syph. 28: 461, 1933).

confirm previously reported findings. The groups of men with the *greatest index of previous (acid) injury* to the skin-site regularly showed a somewhat *greater tendency to develop subsequent (sulfonamide) dermatitis.*

In our subjects there was no correlation between a history of previous systemic exposure to the sulfonamide drugs and the occurrence of dermatitis after their topical application.

CLINICAL FEATURES OF SULFONAMIDE DERMATITIS

The period between the first application of the sulfonamide and the first appearance of dermatitis varied between one and twenty-six days. However, in 48 per cent of the cases the dermatitis became apparent in from two to eight days. This was in accord with the experience of Tate and Klorfajn.[15] However, when a second course of treatment with sulfonamide resulted in a second attack of dermatitis, the reaction time in such a previously sensitized subject was never more than one or two days.

There were 13 men who had two or more courses of sulfonamide treatment and developed dermatitis. In no case did a dermatitis occur after the first course without subsequent development of dermatitis after the second course. In five men the first course was followed by a mild dermatitis, while the second was followed by a more severe attack. The tendency for the dermatitis to spread beyond the arms was related to the *severity* of the particular attack, and did not appear more frequently with recurrences.

The earliest manifestation of the dermatitis was the appearance of an erythema surrounding one or both burns. The more severe lesions were characterized by marked redness, vesiculation, induration, and sometimes maceration, edema, and denudation. In most instances this contact dermatitis was confined to the arms. However, in four cases of severe local dermatitis there was a dermatitis of variable intensity on the entire face, arms, and thighs. The lesions were maculopapular and sometimes edematous or eczematous. It appeared to us that this distant appearance of a rash could be attributed to *external* spread of the agent over the skin surface during the ordinary daytime activities or at night while the subject tossed about in his sleep. The alternate possibility of absorption and hematogenous or lymphatic dissemination has, of course, been demonstrated in drug eruptions after topical application; but this did not appear to have taken place in our cases.

In no instance did the dermatitis cause serious illness. The eruption subsided, usually in one or two days, but always within ten days after the ointment was discontinued. After one to two weeks the area was healed, except that in the more serious cases a bluish discoloration persisted for some time.

PATCH TESTS IN SULFONAMIDE TREATED SUBJECTS

All the subjects were patch-tested with the sulfonamide selected for their treatment *before* (usually on the day before) the sulfonamide treatment was begun. On the completion of the treatment, usually when there was complete epithelialization, an "after-treatment" series of tests was done with all four sulfonamide ointments. This "after-treatment" series was repeated at three-

to four-week intervals for as long as the subject was available. Results of these "after-treatment" patch tests for the men who developed dermatitis are given in detail in Tables V and VI.

Of the 49 subjects who had developed differing degrees of clinical dermatitis after application of the sulfonamides, 31 per cent had positive patch tests. *All* the men who had *severe* dermatitis gave positive patch tests. The paucity of positive patch tests in the sulfathiazole and sulfadiazine groups is probably due to the absence of severe dermatitis in these groups. Of course the patch tests were performed on the back, distant from the areas of previous dermatitis on the arms. It is therefore quite conceivable that the sensitivity of the previously affected sites was not brought into evidence by the patch tests. Perhaps a far greater number of positive reactions would have been elicited by patch tests at the healed areas of previous dermatitis.

Only 10 per cent of all the subjects had positive patch tests exclusively to the sulfonamide used in treatment; all others showed some degree of *cross reaction* to related sulfonamide compounds. Table VI shows the distribution of cross reactions with different chemical radicals and other compounds related to the sulfonamides used. The results indicate the complexity of the sensitizations and indicate that *different individuals can be sensitized by the same compound in different ways, i.e., to the different configurations or parts of the sensitizing molecule.*

Eight men had positive patch tests after treatment, though they had *not* developed a clinical dermatitis. Four of these had the positive reaction to sulfonamides with which they had not been treated.

It was difficult to evaluate the significance of the mild or (+) reactions. They appeared rarely in the presence of a severe dermatitis, but did appear more often in men with slight involvement. This would seem to establish some importance to that result. The (+) readings appeared most often as crossed reactions or in cases where there was no clinical dermatitis. While we have included this type of response to the patch test (faint, irregular erythema) in the tables, we have not considered it a proof of hypersensitivity and *have not evaluated it as a positive test statistically.*

Even disregarding the (+) reactions, a total of 16 men showed definite cross sensitization, i.e., (+) or stronger reactions, to sulfonamides other than those with which they had been treated. Some of these men exhibited multiple cross sensitizations, giving a total of 23 crossed positive patch test reactions (Table V). Of interest in Table V is that the two groups which gave the least dermatitis, i.e., the sulfadiazine-treated and sulfathiazole-treated groups, gave a relatively larger incidence of cross sensitization in men who developed no clinical dermatitis. The greatest sensitizer, sodium sulfadiazine, apparently caused little cross sensitization, while the more weakly sensitizing sulfanilamide apparently caused a good deal. The frequency with which the four sulfonamides *elicited cross reactions when used as testing agents* was about equal.

It was thought possible that cross sensitization might have been influenced by exposure to the same or other sulfonamides before the subjects came into our

TABLE V. PATCH TESTS IN SUBJECTS TREATED WITH VARIOUS SULFONAMIDE CREAMS

DERMATITIS			AFTER-TREATMENT PATCH TESTS					ONE PLUS OR GREATER CROSS-REACTIONS TO:			
DEGREE	NO. OF MEN	0	(+)	+	++	+++	TOTAL POSITIVE*	SODIUM SULFADIAZINE	SULFADIAZINE	SULFANILAMIDE	SULFATHIAZOLE
Group A: 49 Subjects Treated With 5% Sodium Sulfadiazine											
Severe, bilateral	5	0	0	2	2	1	5	–	2	0	0
Moderate, bilateral	2	2	0	0	0	0	0	–	0	0	0
Slight, bilateral	14	10	4	0	0	0	0	–	0	0	1
Slight, one arm	7	2	4	1	0	0	1	–	0	0	0
No Dermatitis	21	13	6	2	0	0	2	–	0	0	0
Totals	49	27	14	5	2	1	8	–	2	0	1
Group B: 54 Subjects Treated With 5% Sulfanilamide											
Severe, bilateral	5	0	0	0	3	2	5	1	3	–	3
Moderate, bilateral†	4	1	0	0	2	1	3	0	1	–	2
Moderate, one arm	1	1	0	0	0	0	0	0	0	–	0
Slight, one arm	2	1	1	0	0	0	0	0	0	–	0
No Dermatitis	42	42	0	0	0	0	0	0	0	–	0
Totals	54	45	1	0	5	3	8	1	4	–	5
Group C: 72 Subjects Treated With 5% Sulfathiazole											
Moderate, bilateral	1	1	0	0	0	0	0	0	0	0	–
Slight, bilateral	1	1	0	0	0	0	0	0	0	0	–
Slight, one arm	3	0	3‡	0	0	0	0	0	0	0	–
No Dermatitis	67	65	2	0	0	0	0	3	2	0	–
Totals	72	67	5	0	0	0	0	3	2	0	–
Group D: 79 Subjects Treated With 5% Sulfadiazine											
Slight, bilateral	1	0	0	0	1	0	1	1	–	1	1
Slight, one arm	3	3	0	0	0	0	0	0	–	0	0
No Dermatitis	73	69	4	0	0	0	0	1	–	1	0
Totals	77	72	4	0	1	0	1	2	–	2	1

*Only + or higher listed in this column.
†One subject had + to Sulfanilamide on preliminary test; ++ on after-treatment test.
‡One subject had the (+) on preliminary test.

TABLE VI. REACTIONS TO PATCH TESTS IN EIGHT SUBJECTS, SHOWING CROSS REACTIONS TO CHEMICAL GROUPINGS AND RELATED COMPOUNDS

SUBJECTS TREATED WITH	DERMATITIS	RESULTS OF PATCH TESTS WITH:								
		SODIUM SULFADIAZINE	SULFANILAMIDE	SULFATHIAZOLE	SULFADIAZINE	SULFANILIC ACID	SULFAPYRIDINE	SULFAGUANIDINE	PARA AMINO BENZOIC ACID	PROCAINE
1. Sulfanilamide	Severe bilateral	0	++	(+)	+	0	0	0	0	(+)
2. Sulfanilamide	Severe bilateral	0	++	++	0	0	0	0	0	0
3. Sulfanilamide	Severe bilateral	0	++	0	0	(+)	0	0	0	?
4. Sulfanilamide	Moderate bilateral	+	++	(+)	+	++	0	+	+	+
5. Sulfanilamide	Moderate bilateral	0	+++	++	+	0	0	0	0	0
6. Sulfadiazine	Slight bilateral	+	+	+	++	++	+	+	+	0
7. Sulfadiazine	No dermatitis Positive Patch	+++	0	0	(+)	++	0	?	+?	0
8. Sulfadiazine	No dermatitis	+	+	++	+	+++	0	0	0	0

studies. The figures, however, indicated that this was not true. They are as follows:

8 subjects showing *no* cross sensitization:
history of sulfonamide exposure, 3; no history, 5
15 subjects showing cross sensitization:
history of sulfonamide exposure, 6; no history, 9

Reports on cross or group sensitization have varied in the literature. Tate and Klorfajn[15] reported that all the cases which they patch tested gave positive reactions to sulfanilamide, sulfapyridine, and sulfathiazole although treatment was with only one of these (usually sulfanilamide).

ORAL ADMINISTRATION OF SULFADIAZINE IN SENSITIZED AND NONSENSITIZED INDIVIDUALS

A study was conducted to ascertain how the previous external exposures to one of the sulfonamide creams and how the cutaneous sensitization produced by such external exposures would influence the subjects' reaction to subsequent *oral administration* of a sulfonamide. For this purpose sulfadiazine tablets were administered to three groups of selected men as follows: 3 Gm. were administered one dose, and 1 Gm. every three hours until a total of 6 Gm. had been administered. Equal amounts of sodium bicarbonate were given with the sulfadiazine. The selected groups were as follows:

Group 1 consisted of 12 men who had developed a varying degree of dermatitis after the local application of the various sulfonamide creams to the arms. Group 2 consisted of 31 men to whose arms one of the four sulfonamide creams had been repeatedly applied *without* the subsequent development of dermatitis. Group 3 was a series of 13 men without known previous exposure to any of the sulfonamide drugs. These men were used as controls.

The results were as follows:

In Group 1, eight of the 12 men (66 per cent) experienced a reaction to the oral administration of sulfadiazine. Three of the four who did not react to oral sulfadiazine had had only slight dermatitis and had not had a positive patch test. The reactions consisted of a generalized papulomacular, pruritic eruption over the trunk, arms, and thighs in five men. Two had only a recrudescence (flare-up) of the previous *local* dermatitis in the arms; and one developed considerable chilliness and general itching with no visible rash.

In Group 2, five of the 31 men (16 per cent) developed slight chilliness, nausea, headache, dizziness, or chills during the oral administration of sulfadiazine. *None of the men developed a rash.*

In Group 3, there was no instance of any untoward effects from the oral administration of sulfadiazine.

Tate and Klorfajn[15] reported that in all but three cases, those tested with oral administration of the same or another sulfonamide, resulted in flare-ups of the dermatitis. These three exceptions were in patients with negative patch tests. Park,[18] in his series of sulfanilamide treated and sensitized cases, administered sulfapyridine orally to six of his men and sulfaguanidine to six.

In each group, four subjects gave a reaction. Fisher[19] also found that of eight patients who developed dermatitis from local use of sulfanilamide, seven had a skin reaction (four generalized, three flare-ups of original area) after oral administration of the same drug.

It would appear from our results that *an individual strongly sensitized by the topical application of any sulfonamide runs a considerable risk of cutaneous and other untoward reactions upon subsequent administration of this group of drugs by mouth.*

According to recent reports, desensitization or hyposensitization may sometimes be accomplished both when the patient has been sensitized orally[20] and when sensitized by topical application.[18, 21] But, even if successful, the process is long and tedious, and it seems likely that the state of desensitization or hyposensitization is not permanent.[18, 22]

SUMMARY

1. A total of 254 men was divided into four groups, and each group was treated with a cream containing one of four sulfonamides. These creams were repeatedly applied in the treatment of standard burns on both arms.

2. Of 49 men treated with 5 per cent sulfadiazine cream, 57 per cent developed a dermatitis.

3. Of 54 men treated with 5 per cent sulfanilamide cream, 22 per cent developed a dermatitis.

4. Of 72 men treated with 5 per cent sulfathiazole cream, 7 per cent developed a dermatitis.

5. Of 79 men treated with 5 per cent sulfadiazine cream, 5 per cent developed a dermatitis.

6. In 48 per cent of the cases which developed dermatitis, the eruption appeared within two to eight days after the commencement of the local applications.

7. Only in the sodium sulfadiazine series did there seem to be a significant direct relationship between the number of applications and the incidence of dermatitis. Perhaps the size of the exposed area was also a factor here.

8. There seemed to be a direct relationship between the degree of previous skin damage at the site exposed to the sulfonamide and the incidence of sensitization.

9. Once a man developed a clinical sensitization dermatitis, he could not be treated again without again developing a dermatitis.

10. Only 31 per cent of the men who developed some degree of clinical dermatitis gave a positive reaction to a subsequent patch test with the offending sulfonamide. But all men who had a severe clinical dermatitis developed such positive patch tests.

11. Only 10 per cent of the men with clinical dermatitis had a reaction exclusively to the sulfonamide used in treatment. In the others with dermatitis there was evidence of cross sensitization to other sulfonamides and to chemical radicals or substances related to sulfonamides.

12. Of 12 men *who developed* clinical dermatitis after topical application of sulfonamide cream, eight developed dermatitis and one general symptoms and itching upon the subsequent oral administration of 6.0 Gm. of sulfadiazine.

13. Of 31 men who received repeated applications of sulfonamide cream but did *not develop* a clinical dermatitis, five suffered from general symptoms but *no* dermatitis when later given 6.0 Gm. of sulfadiazine by mouth.

14. Of 13 control subjects who had had no known previous exposure to sulfonamides, none developed dermatitis or general symptoms from the same oral dose of sulfadiazine.

CONCLUSIONS

1. As regards the incidence of sensitization on topical application, the sulfonamides tested can be arranged in increasing sensitizing potential; and this order corresponds to the increasing solubility in water:

Group I
1. Sulfadiazine
2. Sulfathiazole

Group II
3. Sulfanilamide
4. Sodium sulfadiazine

2. Cross sensitization to other related compounds occurs with significant frequency after exposure to a sulfonamide.

3. Preceding superficial skin damage at the site of topical application is a powerful influence in increasing the incidence of sensitization by externally applied sulfonamides.

4. Previous exposure to and/or skin sensitization by externally applied sulfonamides materially increases the risks of cutaneous and general reactions on subsequent oral administration of the same or related drugs.

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EFFECTIVE TREATMENT OF EXTREMELY SENSITIVE HAY FEVER PATIENTS BY THE CHILLING METHOD*

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THE disadvantages of treating hay fever patients with injections are due in many instances to the too rapid absorption of the antigen, especially in those most sensitive. In this study we tried to find a means of diminishing the rate of absorption.

Many attempts have been made to slow the absorption of pollen extracts by the addition of various substances known to cause delay, such as lanolin and olive oil,[1] almond oil,[2] glycerol,[3, 4] formalin,[5] gelatin,[6, 7] and alum.[8] Some of these are irritating, unstable, or nonantigenic. The inconvenience of administration is due to the following facts: the oily extracts must be given intramuscularly with a long needle; the gelatin extracts must be liquefied in warm water or allowed to remain at room temperature before use. If a constitutional reaction should occur after the administration of one of these extracts, it is likely to appear one hour or more after the injection. At this time the patient would no longer be at the physician's office, and adequate help may not be at hand. The majority of the allergy clinics now use the standardized aqueous ragweed extracts exclusively.

The principle of chilling is presented as a new approach to solve the problem of slow absorption of allergenic extracts. The retardation of absorption following vasoconstriction due to chilling should enable the dosage of ragweed extract to be increased, and in this way give more effective clinical results. This is especially important in the most sensitive ragweed cases which do not tolerate effective dosages with the regular type of therapy.

The chilling method has been used in the retardation of absorption of some injected drugs, such as penicillin, causing a longer maintenance of adequate blood levels of the drug. Trumper and Hutter[9] chilled the deltoid muscle for two hours before and twelve hours after an injection of 50,000 units of penicillin. This procedure maintained bacteriostatic levels for twelve hours and gave a 50 per cent saving in the total amount of penicillin required for each patient.

In our study, a small ice bag supported by a bandage was applied to the mid-outer part of the arm for fifteen minutes prior to and for fifteen minutes following the subcutaneous injection of ragweed extract to retard the circulation in and around the site of the injection.

Thirty patients, eight males and 22 females within the age group of 14 to 63 years, with late hay fever symptoms were treated with the chilling method during the summer of 1945. In this group, 26 belonged to class *A* and showed a marked reaction on skin testing (intracutaneously) with 10 protein nitrogen units of ragweed extract, four belonged to class *B* and showed a marked reaction on skin testing to 100 protein nitrogen units.

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The tolerance of a patient to an increased amount of injected ragweed extract following an ice bag application was roughly measured by the ratio of time interval from the moment of testing (with 10 protein nitrogen units of ragweed extract) to the moment of the appearance of a marked reaction on the skin without and then with an ice bag.

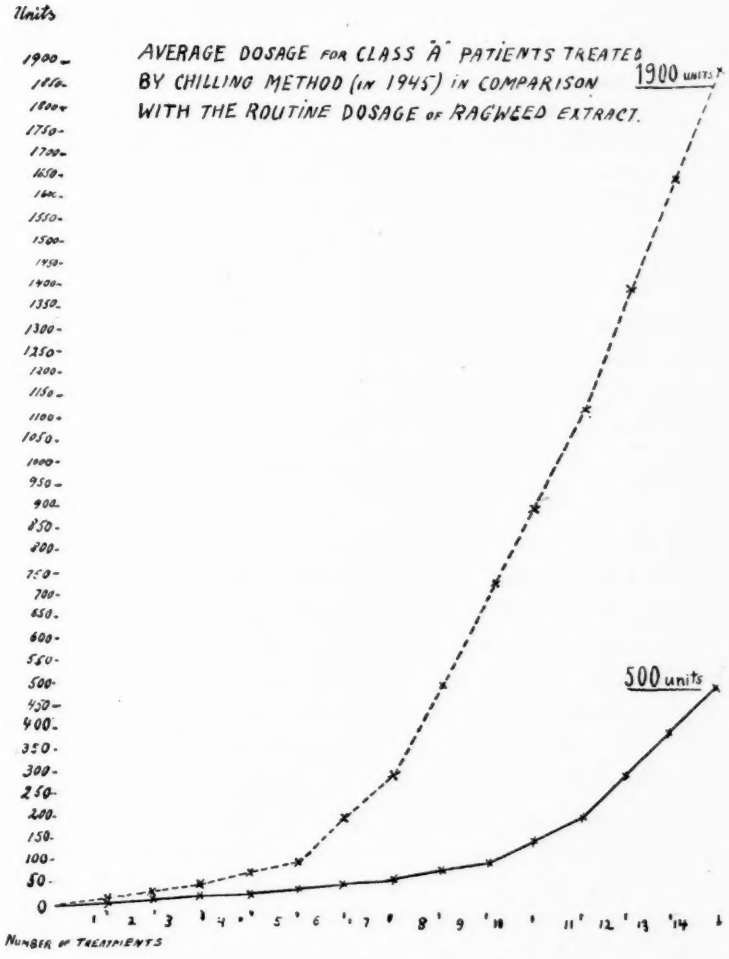
Units
AVERAGE DOSAGE FOR CLASS "A" PATIENTS TREATED BY CHILLING METHOD (IN 1945) IN COMPARISON WITH THE ROUTINE DOSAGE OF RAGWEED EXTRACT.
1900 units
500 units
NUMBER OF TREATMENTS

Fig. 1.

The time interval from the moment of testing to the moment of the appearance of the first signs of a pseudopod-like wheal ranged from four to twenty-two minutes in patients without an ice bag and from six to forty-five minutes in patients in whom an ice bag was applied prior to the testing.

Twenty-five patients of class *A* sensitivity showed a marked skin reaction to 10 units of ragweed extract in an average of six minutes, whereas the same patients showed first signs of a marked reaction in an average of twenty minutes when an ice bag was applied for ten minutes prior to testing.

The ratio of 20:6 was roughly speaking the quotient of the patient's tolerance to an increased dosage. This quotient ($\frac{20}{6} = 3.3$) was multiplied by 500 units which is the usual top dose for class *A* patients. Thus 3.3 × 500 units = 1,650 units, which is the approximate top dosage for class *A* patients when treated with chilling method.

The patients under study attended the clinic on an average of once weekly. The maximum dose given to class *A* ragweed-sensitive patients amounted to an average of 1,900 units of ragweed extract (Fig. 1).

TABLE I. MAXIMUM DOSE OF RAGWEED EXTRACT GIVEN TO CLASS *A* PATIENTS

CASE	TOP DOSE IN UNITS	CASE	TOP DOSE IN UNITS
1	3500	13	2000
2	2750	14	1750
3	2000	15	2750
4	700	16	2500
5	2500	19	2250
6	2500	21	3500
7	1000	22	800
8	1000	23	800
9	2500	24	1000
10	1000	26	2000
11	900	29	2000
12	2250		
(Average 1,900 units)			

By use of the chilling method dosages far in excess of those reached by the routine treatment of hay fever were attained. Although 26 of our patients belonged, according to their skin sensitivity and clinical tolerance, to class *A*, they received in most cases a class *B* dosage. It was impossible to adopt a uniform schedule in all cases for administration of ragweed extract during this study. The degree of dosage-increase was determined in each patient depending upon the degree of local reaction present at the site of injection.

The majority of the patients tolerate a steady increase in dosage. In some the doses were repeated but seldom reduced. A total of 520 chilling treatments rendered to 30 patients in 1945 resulted in seven mild constitutional reactions in the form of hives and itching and five marked reactions which necessitated the use of epinephrine. The number of constitutional reactions (approximately 1 per cent) seems to be low, considering that this group of patients was chosen especially for its high degree of sensitivity.

In a case with grass and ragweed sensitivity, classified as Timothy *B*, Plantain *B*, and Ragweed *A*, we were able by using the chilling method for ragweed alone not only to treat the patient with ragweed extract according to the schedule of *B* cases, but succeeded in increasing the dosage of ragweed above the dosage schedule for timothy and plantain class *B* cases.

One-half of the patients belonging to class *A* who had attained a top dosage above 2,500 units were placed on a perennial basis with treatment once every four weeks. Some of them are receiving a dosage from 4,000 to 5,000 units.

The routine treatment of hay fever patients with an *A* class sensitivity requires about 14 visits to reach the top dose which is about 500 units. In this study we were able to reach 500 units after eight to nine treatments, which saves about 40 per cent of the number of the visits to the clinic.

During the 1945 hay fever season patients belonging to class *A* and treated with the chilling method had an average of three severe days of hay fever and nine to ten moderate days of hay fever. The number of days free from hay fever symptoms was much higher than in previous years. All patients questioned about the new treatment, without exception, were enthusiastic about the clinical results of treatment and expressed no objections.

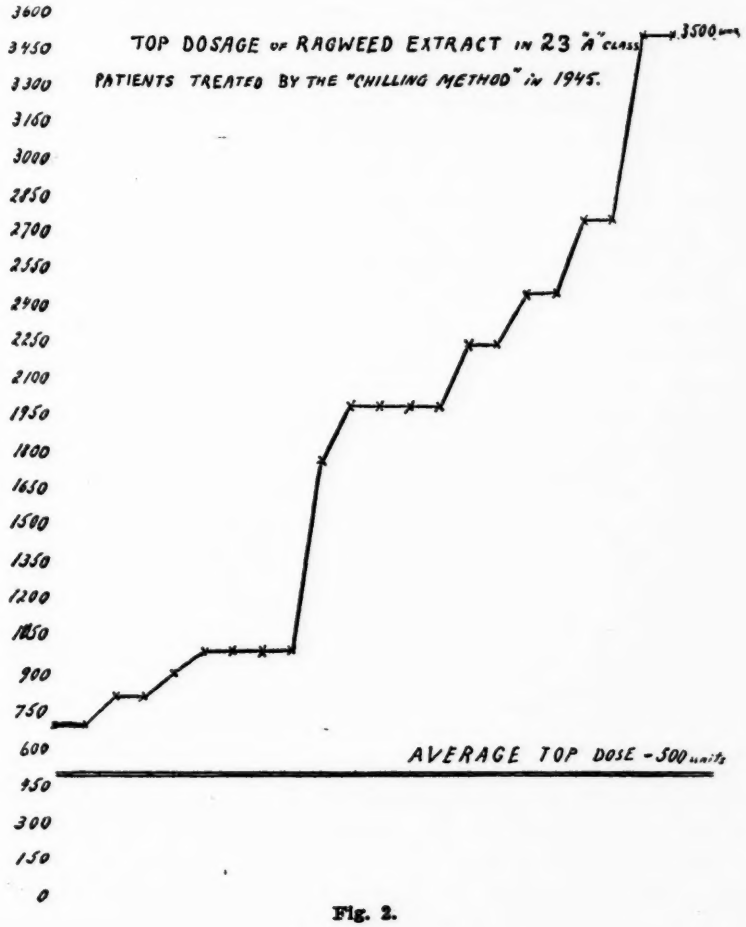

Fig. 2.

Skin testing with a slow absorbing antigen usually caused an increase in the size of the wheal or gave at least a wheal identical in size to that produced by aqueous ragweed extract, due to a prolonged contact between the antibody and antigen. A wheal of diminished size was obtained regularly upon testing sensitive persons by the chilling method when compared to the wheal obtained after

testing with identical extract, without chilling. Twenty-three ragweed-sensitive cases showed a more marked skin test to the aqueous ragweed extracts by the routine intracutaneous method than to the same extract when the intracutaneous test was performed along with the chilling technique. The smaller and delayed skin reaction on testing by the chilling method, the possibility of treating patients with much higher doses, practically without local or systemic reactions, and the more effective clinical results may be explained, besides by the slow absorption, by some "deactivation" of the antigen by the chilled tissues.

The poor local circulation (anemia) in the chilled tissues may play a role in this "deactivation."

SUMMARY

1. The principle of chilling was utilized in the treatment of class *A* patients (the most extremely sensitive) suffering from late hay fever.

2. Thirty hay fever patients were treated with dosages exceeding three to four times the routine dosage of ragweed extract due to the retardation of absorption following the vasoconstriction of chilling. The chilling method does not require a reduction in dosage when intervals between injections are two to three weeks. One-half of the treated patients belonging to *A* class were able to receive perennial treatment following a course of treatment with chilling method. The dosage can be increased even during the hay fever season.

3. The chilling procedure is timesaving, since it diminishes the number of visits by 40 per cent.

4. Constitutional reactions occurred very seldom, and no delayed reaction was observed.

5. The clinical results of treatment gave marked improvement with effective relief of symptoms of hay fever in all treated patients.

6. Chilling renders the injections painless.

I gratefully acknowledge my great indebtedness to Dr. W. C. Spain and Miss M. B. Strauss for their helpful suggestions and advice in the preparation of this paper.

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PENICILLIN IN INTRACTABLE ASTHMA

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THIS brief communication is designed to summarize the present status of penicillin as a therapeutic agent in the treatment of asthma.

With the introduction of new methods of treating any given ailment, there is always an initial phase of confusion during which innumerable reports pour in, some of them wholly favorable, some doubtful, some decidedly unfavorable. The use of penicillin in so-called "intrinsic" asthma has been no exception, and I shall attempt to evaluate its status in the light of present available information.

REVIEW OF LITERATURE

In January, 1945, there appeared an article by Schonwald and Deppe[1] who suggested that the allergic effect of an occult bacterial focus might be lessened or eliminated by the use of penicillin.

These authors identify intrinsic allergy as a type in which the causes are considered to be within the body and are attributed to bacterial sensitivity, while extrinsic allergy is that due to causes entering the body from without. For the purpose of our present discussion, therefore, we shall accept the term, intrinsic asthma, as synonymous with bacterial asthma.

Schonwald and Deppe[1] treated 86 allergic patients. Of these cases, 21 were definitely of the intrinsic variety and 65 were mixed allergies. They have been listed as follows: asthma 56, asthma complicated 13, migraine 6, rhinitis 10, and gastrointestinal allergy 1. At the time of their writing 16 patients had shown no improvement with penicillin, 35 were moderately improved, 34 were markedly improved, i.e., they were free or almost free of symptoms.

In February, 1945, Sweeney, Morginson, Robinson, and Kilpatrick,[2] in a general study of the use of penicillin, included seven cases of asthma. These cases were thoroughly studied and were thought to be bacterial in origin. They were treated with doses of from 1,000,000 to 5,000,000 units of penicillin intramuscularly over an average period of fourteen days. Initially, there seemed to be some evidence of improvement, but it was not sustained.

In April, 1945, Hampton, Wine, Allen, Thompson, and Starr[3] reported nine cases of asthma treated with penicillin. Although eight showed definite reactions to extrinsic factors, control of these factors failed to alter the asthmatic state of any of these patients. It was assumed that the etiology was probably a primary bronchial infection, and for that reason treatment with penicillin was instituted.

Five patients received continuous therapy of a total of 500,000, one patient 800,000, and one patient 1,300,000 Oxford units intramuscularly. Eighty thousand to 100,000 units were administered each twenty-four-hour period at four-hour intervals. At a later date the seven patients having previously received intramuscular penicillin and two additional patients were treated with penicillin intratracheally by means of a pressure spray and a curved cannula extending to the base of the protruded tongue. Eight of these received a total of 500,000 units, and one received only 180,000 units because of the precipitation of asthma at the time of each administration of penicillin.

In conclusion, these authors[4] stated that there was slight clinical improvement in four of the nine cases of intrinsic bronchial asthma treated with penicillin, as evidenced by the occurrence of fewer and less intense symptoms and physical signs requiring emergency measures and by vital capacity studies. It was the writer's opinion, however, that

TABLE I. SUMMARY OF CASES TREATED WITH PENICILLIN INTRAMUSCULARLY

CASE	AGE (YR.)	DURATION OF ILLNESS (YR.)	EXTRINSIC FACTORS	OTHER ACTIVE THERAPY	TOTAL DOSE (OXFORD UNITS)	RELIEF DURING THERAPY	RECURRENCE OF SEVERE ASTHMA	SUBSEQUENT RELIEF	INTERVAL SINCE THERAPY (MO.)
L. R. S.	58	5½	Present	Autogenetic vaccine	1,000,000	Excellent	None	Moderate	7
W. A.	44	6½	None	Bronchoscopy; autogenetic vaccine	2,125,000	Moderate	1 week	None	7
C. C.	48	7	Present	Bronchoscopy; autogenetic vaccine; dust pollen	2,000,000	Moderate	1 month	None	11
P. F.	21	8	None	Autogenetic vaccine	1,500,000	Moderate	None	Moderate	8
J. G.	44	35	Present	Pollens; dust; bronchoscopy; autogenetic vaccine	760,000	None	Continuous	None	8
C. S.	26	5	Present	Bronchoscopy; autogenetic vaccine; pollens	1,000,000	Excellent	None	Excellent	18
C. M.	31	18	None	Bronchoscopy; autogenetic vaccine; tonsillectomy	1,000,000	None	Continuous	None	18
M. R.	47	18	Present	Pollens; dust; autogenetic vaccine	1,000,000	Excellent	4 months (transient)	Excellent	14
L. W.	69	5	None	Bronchoscopy; autogenetic vaccine	1,000,000	None	Continuous	None	18
E. J.	31	9	Present	Pollen; bronchoscopy; autogenetic vaccine	1,000,000	Moderate	7 months	None	13
V. C.	20	14	Present	Bronchoscopy; autogenetic vaccine	1,000,000	Excellent	None	Excellent	13
M. G.	35	7	None	Bronchoscopy; autogenetic vaccine	1,000,000	Excellent	3 months	None	19
W. M.	69	12	Present	Autogenetic vaccine	1,000,000	Excellent	2 months	None	19
S. H.	49	40	Present	Dust; bronchoscopy; autogenetic vaccine	1,000,000	Moderate	1 month	None	20
E. T.	37	12	Present	Nasal surgery; dust; pollens	1,000,000	None	Continuous	None	21
J. N.	70	16	None	Autogenetic vaccine	1,000,000	None	Continuous	None	8
C. C.	34	29	Present	Dust; bronchoscopy; autogenetic vaccine	2,500,000	Moderate	Continuous	None	8
C. T.	20	17	Present	Bronchoscopy; autogenetic vaccine	1,000,000	Moderate	None	Moderate	6

TABLE I.—CONT'D

CASE	AGE (YR.)	DURATION OF ILLNESS (YR.)	EXTRINSIC FACTORS	OTHER ACTIVE THERAPY	TOTAL DOSE (OXFORD UNITS)	RELIEF DURING THERAPY	RECURRENCE OF SEVERE ASTHMA	SUBSEQUENT RELIEF	INTERVAL SINCE THERAPY (MO.)
L. S.	33	5	None	Nasal surgery	1,000,000	Moderate	None	Moderate	6
P. J. S.	64	60	Present	Pollens; bronchoscopy; autogenetic vaccine	1,000,000	None	Continuous	None	12
V. P. E.	59	4	Present	Pollens; bronchoscopy; autogenetic vaccine	1,000,000	Excellent	12 months	Moderate	18
E. Y.	34	4	None	Autogenetic vaccine	1,500,000	Moderate	None	Moderate	4
W. K.	22	20	Present	Dust; nasal surgery; autogenetic vaccine	1,200,000	Moderate	None	Moderate	5
G. A.	55	5	None	Bronchoscopy; autogenetic vaccine	1,000,000	None	Continuous	None	7
J. D.	9	4	Present	Pollens; bronchoscopy; autogenetic vaccine	1,000,000	Excellent	None	Excellent	18
P. H.	4	1½	Present	Bronchoscopy; autogenetic vaccine; T & A	1,000,000	Excellent	6 months	Moderate	8
T. M.	8	7	Present	Dust; bronchoscopy; autogenetic vaccine; T & A	1,000,000	Excellent	6 months	Moderate	12
J. A.	66	15	Present	Bronchoscopy; autogenetic vaccine; dust	1,500,000	Moderate	Continuous	None	18
M. F.	75	30	Present	Bronchoscopy; autogenetic vaccine; dust	1,000,000	Excellent	10 months (transient)	Excellent	12

penicillin administered intramuscularly or intratracheally in the dosages used in this study was of little or no value, and that penicillin offers no advantage over other types of therapy in the treatment of intrinsic bronchial asthma.

In April, 1945, Barach and his co-workers[4] reported an interesting series of cases dealing with the inhalation of penicillin aerosol. Included in their series were twelve cases of asthma which were of the intrinsic variety. Penicillin aerosol was administered in daily doses of from 100,000 to 250,000 units, and over periods of time from five to twelve days. Clinical improvement of moderate degree occurred in eight of the twelve cases. In four of the improved cases, recurrence of symptoms took place within a period of approximately one month. Of these four cases, two were given second courses of therapy; one responded favorably, and one not at all.

Significant results of Barach and associates'[4] series were that predominating organisms in the sputum cultures were consistently absent twenty-four hours after discontinuance of treatment, and that satisfactory blood levels were obtained by the inhalation route. They pointed out that the aim of treatment is generally not a high blood level, but a local application of penicillin on the bronchial wall.

Leopold and Cooke[5] reported two cases of bacterial asthma treated by intramuscular penicillin. Both showed marked and lasting improvement. They alluded to 25 other cases under observation in which the results may have been not so favorable.

Vermilye,[6] in a widespread use of aerosol penicillin in a variety of conditions, stated that this method has been "very successful in 25 cases of bronchial asthma, a number of which also showed extrinsic allergies to foods, pollens, or danders." Only two of these cases are described. He suggests the advisability of continuing therapy in diminishing amounts after the initial period of therapy, and the maintenance of daily prophylactic doses for a number of weeks.

SUMMARY OF 29 CASES

Our own brief series includes 26 adults and three children (Table I). Every case had been thoroughly studied from the allergic standpoint and every effort made to control extrinsic factors or to hyposensitize the patients to them. Each case was adequately surveyed by a competent otolaryngologist and all indicated surgical or other therapeutic procedures had been executed. Twenty of these cases, including the three children, had been bronchoscoped and treated with autogenous vaccine prepared from the aspirated secretions. Although all cases had been followed for one year or more, none had shown satisfactory progress. In each instance, therefore, it was thought justifiable to treat the patient with penicillin intramuscularly, assuming intrinsic factors to be of paramount importance.

During the administration of penicillin, all cases were hospitalized and control of extrinsic factors maintained. The penicillin was administered at three-hour intervals in doses of 12,500 or 25,000 units. The average total dose was 1,300,000 Oxford units, most cases receiving 1,000,000 units.

Eleven of the 29 cases showed an excellent response while receiving penicillin, i.e., they became symptom free or so nearly so that they required no symptomatic therapy. Eleven cases showed improvement, but continued to require occasional, although less frequent symptomatic treatment. Seven cases showed no change whatever either during the hospital course or thereafter.

Of the eleven cases showing excellent response, seven continued to do very well thereafter, and four had moderate symptoms but of much lesser degree than formerly. Seven of these eleven experienced acute exacerbations at intervals of one to twelve months with an average of six months. Four of these recurrences were again treated and again recurred. A fifth one of the recurrent cases subsided spontaneously in thirty-six hours and has had no further trouble during ten months. This particular patient had previously experienced no comparable relief in many years. The remaining two recurrent cases received no further penicillin, and continued with periodic exacerbations which were not as severe or prolonged as those before penicillin therapy.

Of the eleven cases who did just moderately well during the period of administration, five have continued at a lower level of symptomatology, although requiring occasional symptomatic medication, and six relapsed immediately to their previous status.

It is nearly impossible to evaluate accurately such a series from a statistical angle. Of 29 cases, four (including one of the three children) have

remained well for over one year, and a fifth has had only one transient recurrence in the same time. Nine cases have seemed to show definite improvement although not complete freedom from symptoms. Fifteen cases showed no benefit whatever insofar as their subsequent courses were concerned.

DISCUSSION

In attempting to evaluate the information presented, there are a number of important factors to be considered.

First of all, intrinsic asthma, in many instances, exhibits seasonal fluctuations, being more troublesome in the cold months of the year when respiratory infections are prevalent, and tending to subside somewhat during the warm months. Obviously, therefore, a greater percentage of good results may be seen in cases treated in the spring than in the fall, if only the immediate posttreatment course is considered. Schonwald et al,[1] for instance, reported few failures, but significantly indicated that, as their report was made, several of the cases, definitely intrinsic, and who were cleared during antibiotic therapy, had shown recurrences of symptoms, more or less at the same time when fall weather set in. It would seem wise, therefore, to follow each case for a full year after therapy before drawing any conclusions.

It has been emphasized in our brief series that every case included had had prolonged control of extrinsic factors and careful attention to structural or infectious abnormalities of the respiratory tract. In a few untabulated cases, which were only casually observed, penicillin was administered without study and the results were uniformly poor. This would indicate the advisability of attempting antibiotic therapy only after an adequate appraisal of the case from all angles, and after proper attention has been given to extrinsic factors, correction of structural abnormalities, and institution of adequate drainage of sinuses and bronchi where indicated.

Barach and associates'[4] studies on bacterial flora in the sputum were noteworthy, and I feel that such studies might well be included in any further investigation of this nature. It would seem wise, also, in future work, to consider doing penicillin sensitivity studies on the organisms detected.

Penicillin aerosol shows much promise in many types of bronchial infection; but too few cases of intrinsic asthma, treated by this route, have as yet been reported to afford a comparison with the intramuscular mode of administration. The oral route represents another incompletely evaluated field which may yield interesting information.

SUMMARY

1. The available literature concerning the use of penicillin in intrinsic asthma has been reviewed and summarized. In addition 29 cases treated by the intramuscular route have been reported. The results are somewhat conflicting, but the following conclusions seem justified: (a) Penicillin cannot be considered a panacea for the treatment of intrinsic asthma. It should be regarded, more properly, as a therapeutic agent for the treatment of infectious processes, and, as such, seems to be effective in a small, but worth-while pro-

portion of selected cases of bacterial asthma. (b) The results thus far are not completely conclusive, but are sufficiently promising to warrant further study of the use of penicillin in bacterial asthma.

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ETIOLOGIC EVALUATION OF ASTHMA IN 100 CASES RETURNED FROM OVERSEAS*

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THIS hospital serves to debark and distribute patients who have returned from the European Theater of Operations, which includes North Africa, Italy, and France. It was felt, during 1944, that the number of patients returned from overseas with the diagnosis of asthma was disproportionately high. Investigation showed that asthmatics constituted from 1 per cent to 3 per cent of the total number evacuated to the United States. Since asthma disqualifies a man for induction into the Army, and its detection at a port of embarkation will disqualify any individual for overseas duty, it was felt that the condition was either started, or latent manifestations precipitated while overseas. An investigation of the etiologic factors in 100 consecutive cases was instituted.

PURPOSE

The investigation was undertaken as an attempt to isolate specific etiologic agents or factors which caused or precipitated asthmatic attacks in the patients returned from overseas. Constitutional predisposition was carefully weighed in each case, based on family and personal history. Initial attacks were carefully evaluated with particular regard to the circumstances of onset.

METHODS AND MATERIALS

Standard forms for history and examination were prepared. Each overseas record was carefully examined and abstracted. A complete history, physical examination, x-ray study, sedimentation rate, blood count, and sputum examination were available for each patient. Ear, nose, and throat, dental, and neuropsychiatric consultations were undertaken where indicated. The average hospital record available to us for each patient covered approximately two to three months of illness. Skin tests were done on each patient by the intradermal method employing allergens obtained from the Fourth Service Command,† supplemented by molds from the Massachusetts General Hospital Laboratory,‡ foods, and bacterial vaccines,§ which were prepared in our own laboratory.

The group for study included 94 men and 6 women. The age of onset of the initial attack of asthma is indicated in Table I. The majority of our cases

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†House dust, cat dander, dog dander, horse dander, rabbit dander, sheep dander, chicken, duck, and goose feathers, orris root, cotton seed, kapok, ragweed, timothy, and careless weed.

‡Alternaria, *Aspergillus niger* and *flavus*, hormodendrum, penicillium, monilia.

§Local dust, overseas dust, staphylococcus, hemolytic and nonhemolytic streptococcus, and *Neisseria catarrhalis*.

TABLE I. SIXTEEN PATIENTS WITH ASTHMA AND ASSOCIATED NEUROPSYCHIATRIC DIAGNOSES

AGE (YR.)	RANK	F.H. ALLERGY	P.H. ALLERGY	DATE OF INDUCTION	DATE OF ARRIVAL AT PLACE OF ONSET	DATE OF ONSET	SITE OF ONSET	OTHER DIAGNOSES	POSITIVE SKIN REACTIONS	MAXIMUM BLOOD EOSINOPHILES	ASSOCIATED SYMPTOMS	CASE NUMBER
36	Pfc.	Neg.	Hay fever	Jan. '41	Jan. '44	Mar. '44	England	Anxious state	0	0	Restlessness, headaches, insomnia, asthma, ppt'd by fear	1
39	Pvt.	Neg.	Neg.	Apr. '42	June '43	Oct. '43	England	Anxious state	0	0	Pain in chest, tremor of hands, sweating	2
23	Pvt.	Neg.	Neg.	July '42	July '43	Aug. '43	England	Inadequate personality	Kapok	0	Resentful, antagonistic, uncooperative	3
22	Sgt.	Neg.	Neg.	July '40	July '43	Feb. '44	North Africa	Post concussion	0	0	Blast injury, Sept., 1943; headaches, backaches, and enuresis	4
29	Pfc.	Pos.	Neg.	June '41	July '43	Nov. '43	England	Anxious state	Feather	4	Headaches, insomnia, tachycardia, palmar sweating	5
31	Pvt.	Pos.	Neg.	June '43	July '44	July '44	Italy	Hypochondriasis	0	0	Resentful, uncooperative, short of breath, unable to do light work	6
32	Pvt.	Neg.	Neg.	Apr. '41	May '42	May '42	North Africa	Anxious state	0	2	Nervous, irritable, wheezes, when under tension	7
32	Capt.	Neg.	Neg.	Jan. '32	Feb. '44	Feb. '44	Ship to ETO	Anxious state	0	2	Nervous, irritable, palmar sweating, tense	8
30	Pvt.	Pos.	Neg.	Jan. '41	Mar. '44	July '44	England	Anxious state; vasomotor rhinitis	Feathers	0	Worried, tense, felt that he had tuberculosis	9
27	1st Lt.	Neg.	Neg.	Jan. '41	Mar. '43	Nov. '43	Italy	Hepatitis anxious state	0	0	Started while in hospital for hepatitis, tense, easily fatigued	10
27	Pfc.	Neg.	Neg.	June '42	Dec. '42	Fall '29	North Africa	Reactive depression	Kapok dust	1	Compulsive acts, depression, required confinement to closed ward	11
23	Pvt.	Pos.	Neg.	Jan. '42	Jan. '44	July '44	Italy	Post concussion syndrome	0	8	Tinnitus, insomnia, easy fatigability	12
32	1st Lt.	Neg.	Neg.	Feb. '31	July '42	Nov. '42	North Africa	Anxious state	0	0	Nervous, unable to recall events, tense	13
35	T/5	Pos.	Pos.	Feb. '42	May '44	May '44	Italy	Vasomotor rhinitis; anxious state	Pollens	1	Stuttering, tension	14
19	Pvt.	Pos.	Neg.	July '43	June '44	Aug. '44	France	Mental deficiency	0	1	Mental age 10 years, muttered to himself, fixed stare, dirty	15
27	Maj.	Pos.	Neg.	Oct. '40	June '42	May '44	England	Combat exhaustion vasomotor rhinitis	0	3	Furunculosis, anorexia, insomnia	16

had their initial attack of asthma between the age of 20 to 30 years. Seventy-eight of the 100 were asymptomatic when examined at this hospital and required no further treatment.

RELATION OF TIME OF ONSET TO SKIN TESTS

The patients were subdivided into three groups based on the time of onset of symptoms. Fifty-six per cent of the group developed their initial symptoms overseas (Group A); 14 per cent had had at least one previous attack in civilian life, but had had a latent, asymptomatic interval until their arrival overseas (Group B). The remainder, 30 per cent, had had recurrent, mild attacks in civilian life which had been mild and nonincapacitating, and were presumably asymptomatic at the time of physical examination for induction or overseas duty (Group C). The last group suffered a marked exacerbation of symptoms overseas and was unable to continue duty there, even in a limited duty capacity.

Table III indicates the results of the skin tests according to groups. The groups, by subdivision, become small, but several noteworthy points arise. The highest incidence of positive reactions to extrinsic factors occurs in the members of Groups B and C who had had antecedent attacks of asthma in civilian life. Only one case in Group A gave a positive skin reaction to any of the pollens used in skin testing, while 10 positive skin reactions to pollens were noted in Groups B and C. Both groups were exposed to approximately the same degree of wet, cold, and upper respiratory infection. It is noteworthy that Group C, containing individuals who had previously manifested allergic disease, had a higher incidence of positive reactions to intrinsic agents than Group A, in which more than half had only an allergic predisposition as indicated by a positive family history in one or more of the progenitors (Table II).

These results were accepted as contributory evidence that inhalants were rarely significant, and intrinsic factors significant in only a minority of the 56 patients who developed their initial attack of asthma while overseas. Foods could not be incriminated etiologically from the patients' histories.

The relation of incidence of attacks to any specific foreign environment could not be established, since the duration of stay in any one area was usually very limited.

TABLE II. ANTECEDENT PERSONAL AND FAMILY HISTORIES IN 56 PATIENTS WHO DEVELOPED INITIAL ASTHMATIC ATTACK OVERSEAS (GROUP A)

PERSONAL HISTORY OF ALLERGY			FAMILY HISTORY OF ALLERGY		
	TOTAL NUMBER	PER CENT		TOTAL NUMBER	PER CENT
Negative	41	73	Negative	25	45
Positive, total	15	27	Positive, total	31	55
Hay fever	12	21	Asthma	27	47
Other	3	6	Other	4	8

Forty-one of the 56 patients in Group A had no antecedent personal history of allergy, but 31 had a positive family history. It is noteworthy that the predominant manifestation in the allergic members of the family was

asthma (Table II), which made available to the patient a behavior pattern that could be precipitated by undue stress as a manifestation of neuropsychiatric disease.

Groups B and C, therefore, are composed of individuals who fully satisfy the diagnostic criteria for allergic disease. Group A, however, is not so constituted. The symptoms of only 15 of this group (27 per cent) could be unequivocally ascribed to allergy. In 10 cases (19 per cent) no allergy could be established by personal history, family history, skin test correlation, and laboratory examination. In these 10 cases, the associated neuropsychiatric diagnosis made overseas seemed fully adequate to account for the patients' symptomatology. In the remaining 27 (54 per cent) the relative etiologic significance of allergy, functional disease, and infection could not be adequately separated.

TABLE III. POSITIVE SKIN REACTIONS IN 100 CASES OF ASTHMA ACCORDING TO TIME OF ONSET OF SYMPTOMS

	GROUP A 56 CASES		GROUP B 14 CASES		GROUP C 30 CASES	
	NUMBER	PER CENT OF GROUP	NUMBER	PER CENT OF GROUP	NUMBER	PER CENT OF GROUP
Dust 1:10	20	37	8	71	26	87
Feathers 10 PNU	17	30	5	36	14	47
Pollens 10 PNU, 100 PNU	1	2	2	14	8	27
Vaccines 90 mil.	7	13	2	15	15	50
Molds 1:10	3	6	1	7	2	7
Kapok	0	0	0	0	2	7
Danders 0.001 mg. N.	0	0	1	7	1	3

Group A: Initial attack while overseas.
Group B: Initial attack in childhood, then asymptomatic until arrival overseas.
Group C: Recurrent attacks since civilian life with no latent asymptomatic interval.

AGGRAVATING AND PRECIPITATING FACTORS

Nearly all of the patients felt that their attacks were precipitated by prolonged exposure to dampness. The high incidence of trench feet in other patients evacuated from the same areas bears testimony to the amount of moisture to which these men were exposed. Most stated that dust was an aggravating factor. The great majority felt that prolonged exertion and fatigue were contributing factors. In 18 per cent, an immediate antecedent history of an upper respiratory infection was elicited. Foods and alcohol were felt to play no initiating rôle, and many ingested alcohol with symptomatic relief of their acute symptoms. Change of location seemed to be of significance etiologically only insofar as it meant greater exposure to inclement weather. Animals were rarely encountered. Few would admit that fear was a precipitating or aggravating factor, but many felt that worry facilitated the onset of symptoms. The greatest incidence of attacks was noticed at night after retiring. Occupation was not believed to be significant.

No specific relationship was noted to grasses and trees which are found in the areas occupied by our troops. Exacerbation of symptoms frequently occurred which could not be related to a change of pollen content in the air.

The prolonged dampness militates strong against an airborne distribution of pollen. Attacks persisted despite passage from areas of greater to lesser concentration of pollen. It is felt that pollens were not of paramount importance in the great majority of these cases.

The botanical flora of Italy, Sardinia, Corsica, Southern France, Spain, and the coastal shelf of North Africa are essentially similar.[1] The chief pollens, according to Durham,[2] are the grasses and trees. Minor variations in pollination occur in these areas depending on the latitude and altitude. Broadly, pollination may be divided in these countries into the following seasons:

A. Early Forms: (Before April in Southern Italy and Spain)—usually attributed to trees: willow, poplar, ash, elder, and plum.

B. Spring Forms: (Most widespread)—usually due to *Gramineae,* poa, phleum, bromus, dactylis, lolium, cereals, but also trees: olive, oak, maple, cork, plantains, etc.

C. Estival Forms: Gramineae, maize, cynodon, sorghum, anthoxanthum, chenopodium, amaranthus, rumex, carex, compositae.

D. Autumnal Forms: Amaranthus, chenopodium, canna, compositae, poa.

In England, it is noteworthy "that there is no ragweed or anything that corresponds with it."[2] Trees are uncommon causes of hay fever in the British Isles.[3] The spring and summer group of grasses are the causes of pollen difficulties.

PHYSICAL EXAMINATION AND LABORATORY FINDINGS

All of these patients had inspiratory and expiratory rhonchi as well as other chest manifestations during the course of their disease. However, after their arrival at this hospital, only 22 per cent had any chest signs, and of this group only six had had their initial attack overseas. There were three in this group who had permanent chest changes, such as emphysema, but all of these gave a history of asthma in civilian life. Blood pressure was not elevated in any case. The sedimentation rate (Westergren) was normal in all cases except one which was complicated by atypical pneumonia.

CONSULTATIONS

Eleven per cent showed nasal mucous membrane pictures which were described by the consultant as compatible with or characteristic of allergic rhinitis. Seven had had asthmatic attacks in the United States; all had had nasal symptoms in the United States. Eight per cent of this group had positive smears for eosinophiles, and three cases showed x-ray changes which were interpreted as early bronchiectasis. These three cases had all had asthma since childhood. Neuropsychiatric consultations will be described below. Dental clearance for foci of infection was obtained in all cases.

Table I includes 16 cases of asthma, briefly summarized, who were evacuated from overseas with a primary or secondary diagnosis of psychoneurosis which was established in each case by a neuropsychiatric consultant. Two other cases will be described in greater detail. In these cases, it is noteworthy that only three gave an antecedent personal history of allergy, but seven gave a history of allergy in their family, chiefly asthma.

CASE REPORTS

CASE 1.—Patient was a 26-year-old white woman who came into service in March, 1942, and arrived in Constantine, North Africa, in March, 1943. On Sept. 15, 1943, while on a high clear spot where she had been for two months, she suddenly noted the initial attack of a constricting sensation in her chest, which resolved spontaneously. From then on she had recurrent attacks of constriction associated with wheezing and shortness of breath, which frightened her considerably. She could relate no specific etiology to her attack. She noted particularly that there was very little vegetation around her and no animals. She was hospitalized on Oct. 1, 1943.

Her past history is remarkable only in that she has had very severe dysmenorrhea since early childhood which was not helped by endocrine therapy, and, in 1934, she was hospitalized for a "nervous breakdown for one month." The chief symptoms at that time were weakness, tremors of the hands, loss of weight, and difficulty in breathing (which she could not amplify).

Physical examination revealed a well-developed, well-nourished white woman, who, at the time of examination, showed no signs of asthma. However, on several occasions the medical officer of the day who was called during the course of an acute attack, found rhonchi at the end of inspiration and throughout expiration.

Laboratory studies were noncontributory, and the maximum eosinophiles at any time was four. Skin tests showed slightly positive skin reaction only to orris root.

Course in Army Hospitals: During her period of hospitalization overseas, she had many recurrent attacks but responded poorly to any medication, including adrenalin. In January, 1944, she received almost daily letters from her husband, an enlisted man in the United States whom she had recently wed, stating that he was severely ill. She stated that she felt disgusted with herself, overworked, longed for her husband, and longed to return to the United States. She continued to have attacks chiefly at night, until March 14, 1944, when she was sent to the United States. She had no attacks on board the hospital ship, during her stay at this hospital in South Carolina, and only one mild attack at night, which was ascribed to dampness, during a 25-day sick leave. During her leave, she met her husband, who was on furlough, and both overstayed their leave by nine days. They finally returned to the hospital. She was confined to quarters; her husband was placed in the guardhouse, but was transferred to the hospital prison ward when he suddenly developed hematemesis. It was later determined that he was self-inducing emesis and traumatizing his gums to produce blood. He was confined to a closed neuropsychiatric ward.

She was followed in the Allergy Clinic at this hospital where she stated on many occasions that she was "as tight inside as a drum," and noted that her state of mind and body varied with her husband's tribulations on the Neuropsychiatric Ward. Her asthmatic attacks occurred only at night and chiefly around 10:00 P.M., in her own hospital room. Rhonchi were heard on many occasions at the end of inspiration and throughout expiration. Repeated skin test series failed to identify any intrinsic factor which could be related to her symptoms. She was moved from room to room, and her environment completely cleared of all substances which might act as allergens, with no relief. Her attacks responded to aminophylline in twenty minutes; to adrenalin in twenty minutes; and resolved spontaneously, if untreated, in two hours. On two occasions, she was given saline and responded in twenty minutes. However, when she found that she was getting saline instead of aminophylline, she no longer responded before the two-hour period.

On July 6, 1944, she was seen by the Chief of Neuropsychiatric Section, who made the diagnosis of psychoneurosis, anxiety state, moderately severe.

She was discharged from the service in the early part of August, with the primary diagnosis of psychoneurosis, anxiety state. Her husband was discharged approximately the same time as a neuropsychiatric casualty. They returned to her home in South Carolina. Since her return home, she has had no need for therapy, has rare attacks, and is well adapted to civilian life.

This case illustrates a patient with a background of emotional instability, who responded to a series of unfavorable stimuli, with a characteristic picture of asthma. It is felt that extrinsic antigens played a small part in initiating her symptomatology in view of her protracted stay in the same area for the two months preceding the initial attack and the absence of a new pollinating flora in North Africa at that time. The emotional relationship to her attacks is readily apparent as is the freedom from attack when emotional stimuli are removed. The rôle of intrinsic factors, such as endocrine dysfunction, cannot be evaluated completely but probably play no significant part.

CASE 2.—The patient was a 30-year-old white man who was admitted from overseas with a diagnosis of conversion hysteria following a concussion blast. He entered the service on March 19, 1944, and was sent overseas on Aug. 28, 1944. In September, 1944, he arrived at an English Camp and was alerted for crossing the channel. From Sept. 7, 1944, to Sept. 22, 1944, he crossed France with his outfit in no active combat. His only complaints at that time were increasing nervousness and sleeplessness. He had never had any allergic manifestations, and his family history was negative for allergy.

On Sept. 22, 1944, he entered active combat and started to "feel wheezy," and became very short of breath. He reported on sick call and was told that he was "taking asthma." However, despite his nervousness and respiratory difficulty, he returned to duty because he liked his companions and was desirous of continuing in combat. On Nov. 1, 1944, he suffered a blast injury which rendered him unconscious for several hours. Since then, he has been through the various army hospitals en route to the United States with no alleviation of symptoms. Upon exposure to emotional upsets, which include war motion pictures, the sight of guns or cannon fire, he became markedly "shaky," tense, nervous, apprehensive, short of breath, and then developed inspiratory and expiratory difficulty.

Physical examination revealed a well-developed, poorly nourished white man, who was extremely apprehensive. He had a very marked coarse tremor of his hands on extension and looked away from the examiner, although he was manifestly trying to be cooperative. Expiration was prolonged. His palms were cold and clammy.

Laboratory studies were normal. Skin tests were not done.

It is felt that this case clearly illustrates the effect of the emotions on the production and perpetuation of asthma. Psychotherapy becomes essential in this type of case to arrest the process and prevent the complications of asthma.

Table I lists the pertinent findings in 16 patients who were returned from overseas with the diagnosis of asthma plus neuropsychiatric disease. The neuropsychiatric diagnosis in each case was established by a neuropsychiatrist who saw the patient in consultation.

The group consists of 4 officers and 12 enlisted personnel. It is noteworthy that none of these men had developed incapacitating symptoms despite repeated change of environment in the United States, but that all of these men became unfit for even a limited type of duty overseas. None of the patients developed symptoms of asthma during combat when the individual had no recourse but to fight for his existence; nearly all 14 developed symptoms while engaged in specific precombat preparations while there was time to anticipate the horrors into which they would soon be projected.

Some (Cases 4, 12, 16) developed asthma as part of generalized functional disorder after specific mental insult. In three patients (Cases 6, 7, 14) the rôle of extrinsic dusts was suspected because of the onset of symptoms shortly

after arrival overseas. In these cases it is not possible to determine whether the functional disorder resulted from or produced the asthma. Most of the patients (Cases 1, 2, 4, 5, 9, 10, 12, 13, 15) were in the area overseas for at least one month before the symptoms began. In no case could the symptoms be related to alterations in basic flora. Skin tests were of no aid in etiologic evaluation in these cases. The symptoms of one patient (Case 8) began while at sea and progressed so rapidly that it became necessary to hospitalize him for return to the United States as soon as he arrived in England. The asthma cleared on the hospital ship during the trip home.

One patient (Case 2) had had asthma in civilian life, then remained free until December, 1942, when engaged in final precombat training. In north Africa associated neuropsychiatric symptoms were severe enough to demand confinement to a closed ward.

All were improved by their return to the United States.

DISCUSSION

Asthma has been etiologically classified by Rackemann[4] into 2 types extrinsic and intrinsic. Extrinsic asthma includes those patients who are hypersensitive to a foreign substance in their environment and will manifest asthma upon exposure to it. Intrinsic asthma includes those cases in which the cause of the difficulty lies within the body itself, and includes cases of sensitization from an antecedent bacterial infection, a focus of infection, endocrine disturbances, physical allergy, and electrolytic disturbances. Results of therapy in extrinsic allergy have been universally far superior[5] to the results of therapy in intrinsic allergy. We feel that some of the cases which have heretofore been ascribed to intrinsic allergy are largely caused by psychosomatic factors.

All textbooks on allergy are agreed that emotional manifestations may well be the result of a specific attack rather than the cause. It is also recognized that psychogenic influences may aggravate or perpetuate a given attack. However, in the group of cases presented in this paper, we are concerned with individuals who are exposed to emotional stimuli which are rarely, if ever, encountered in civilian life. It is felt that in this group emotional factors are the chief etiological agents. It is readily conceivable that in other groups who are subject to subliminal environmental, or intrinsic stimuli, psychogenic trauma may render an otherwise inadequate etiological agent effective.

It is not our purpose to suggest that emotions are the chief cause of asthma in civilian or even military life. Obviously, civilians are not exposed to the same intensity of emotional stimulus as soldiers in combat areas. However, it is felt that these same factors operate to a lesser degree. Gradations of emotional stimuli are readily seen in our local command clinic where we are concerned with soldiers who are subject to military rule and duty, but will never enter combat areas. In these people psychotherapy becomes an extremely important adjunct to adequate medical treatment.

Dunbar[6] discusses in detail the psychogenic factors which are related to the production of asthma. He cites evidence for somatic preparation in terms of early respiratory disease in individuals who later develop asthma. He notes

that the incidence of respiratory predisposition is greater in asthmatics than in patients with diabetes or heart disease. French and Alexander[7] report cases who gave vent to emotional conflict by asthmatic manifestations, but that when they started to cry the asthma disappeared. They felt that the asthma was equivalent to a cry of anxiety or rage which had been prohibited or suppressed.

McDermott and Cobb[8] have shown that 37 of 50 cases of asthma had a strong emotional component and that 20 reported emotional factors as the precipitating agents in individual attacks. Thirty showed other neurotic traits. Strauss[9] has shown that the majority of asthmatic children are over-anxious and insecure. Six of eight children had a family history of allergy. Rogerson[10] has shown that removal of children from home environment immediately alleviated asthmatic symptoms. All of his patients had been referred to him from allergy clinics, and specific agents have not been found. He notes that on his cases specific antigens were probably not incriminating, and feels that family conflicts are producing the symptoms.

It is felt that many of the cases included in this report fall into the group of constitutionally predisposed individuals who have had previous chest involvements and superimposed fear of lung diseases. Their attacks may then represent imitations of similar attacks which were usually observed in their own families, and closely mimic asthmatic breathlessness. It is obvious that for this type of patient psychotherapy would be more effective than any type of somatic therapy.

Selye[11, 12] has postulated that many varied stimuli may produce a general diffuse physiologic response which he designates as the alarm reaction. If the stimulus is acute enough and intense enough, a state of shock will result. Minor stimuli may never produce the state of shock but, if repeated, may result in physiologic responses which he designates as countershock.

It is obvious that many different stimuli, such as specific external allergens, emotional upsets, and intrinsic allergens, are all capable of producing a symptom complex which we designate as asthma. When asthma is viewed as a part of a diffuse response of the body to varied stimuli, it is understandable why ragweed, or rage, or staphylococci, or cold may all bring about the same end result. It would be interesting to examine the adrenals in chronic asthmatics for hypertrophy of the cortex which Selye considers indicative of the countershock stage of the alarm reaction.

SUMMARY

1. One hundred cases of asthma in soldiers, evacuated from overseas because of inability to perform duty in an even limited capacity, are reported.

2. They can be divided into three groups:

a. Group A (56 cases)—no history of previous asthma before arrival overseas.

b. Group B (14 cases)—an initial attack in civilian life followed by an asymptomatic period which was terminated by arrival overseas.

c. Group C (30 cases)—recurrent asthma in civilian life which became incapacitating overseas.

3. Seventy-eight of the 100 patients examined were asymptomatic and needed no further therapy upon return to the United States.

4. Etiological factors are discussed.

5. The importance of emotional stimuli is evaluated.

6. The possible reaction of asthma to the alarm reaction is presented.

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Special Report

IMMUNOCHEMISTRY*

A REVIEW INTENDED ESPECIALLY FOR STUDENTS OF ALLERGY

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AT SOME stage of evolution, one or more species of organisms must have made the momentous discovery that a very convenient source of food consists of the tissue of other organisms. Perhaps dead organisms were utilized at first, but it would be but a short step to the consumption of the living. The whole of the animal kingdom depends upon eating plant life in some form or other—directly or indirectly—and thus obtains the protein which it needs to build up its tissues, and the carbohydrates or fat to provide energy for its activity. It is only the green plants and certain other vegetable organisms which can synthesize these substances from inorganic materials and carbon dioxide.

Once the importance and desirability of eating the other fellow was discovered, evolution had the opportunity to take a further step, whereby an animal accustomed to eating plant life could also develop the taste for eating other animals. The new taste might eventually supplant the old vegetarian habits completely. A lion eats no plant life, but nevertheless lives on plants indirectly since its usual food consists of large herbivorous mammals.

It was probably not very late in the history of evolution that another and equally important discovery was made. Instead of simply eating other individuals and thus obtaining nourishment, it is possible for certain organisms to learn to live with or upon other organisms and obtain their nourishment, either by eating portions of the tissues, or by absorbing food which would otherwise be utilized by the host. This may or may not result in the death of the host. Organisms that live upon another organism and depend upon it for source of nourishment are known as parasites. Evolution has operated on the parasite as well as upon other living organisms, and the world as we know it contains many examples of extremely accurate adaptations of the parasite to its host.

While parasites were developing, however, the organisms which they made their prey were, in the evolutionary sense, not idle themselves. Whatever the first forms of life on earth may have been, we find at the present time that all organisms have provided themselves with some membrane or sheath to protect themselves against invasion from the outside. In many cases, skin and various kinds of armor have also been developed. The porcupine has developed a coat of quills which must be very effective in preventing any carnivorous animal from eating him, judging from the results when a dog attempts to attack a porcupine. Quills and other defensive armor would be quite effective against

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large pedatory animals, but less effective against smaller organisms which had become parasites, and which either were from the beginning or had become so small as to be invisible to the eye. Such organisms would be able to find their way into the tissues of the host either through the natural openings of the body, or by actually boring a path, as is done by the free-swimming form of certain parasites, such as *Schistosoma.* This process has gone on to such an extent that it may be safely said that probably no organism of any considerable complexity exists which is not subject, at some stage of its existence, to the attack of some sort of parasite or microorganism.

There are two classes of parasitic organisms of predominant concern to us, which we call bacteria and viruses. Some think the latter may have evolved (by degeneration) from the former. Suffice it to say that both are small organisms, invisible to the eye without artificial aid, which may on occasion invade the tissues of suitable hosts. When such an invasion occurs, we speak of it as an infection.

Now just as organisms develop protective devices such as spines or armor to protect themselves against being eaten, so also have they, in practically all cases that we know about, developed protective devices against parasites, including the tiny organisms, bacteria and viruses. Some of these protective devices consist of relatively impermeable membranes, such as skin and the mucous membranes of higher animals. Others include, apparently, the ability to alter the body temperature to one unsuitable to the would-be invader. There must be a great many other such devices. For just as the parasite is capable, in the course of evolution, of evolving in a manner so as to be able to invade a different host, or more effectively invade the same host, the host is capable of evolving in such a way that it can resist invasion. The production of plant resistance to various plant diseases such as the rust disease of wheat (by artificial selection) is but an imitation of this natural process.

We shall seldom use the word *immune* in the strict sense here. We are mostly interested, not in absolute immunity, but in degrees of refractoriness to infection, and the use of the word *immunity* in this way has become accepted terminology. Immunity is only one of the weapons in the basic struggle for life, and denotes the *resistance* which each organism offers against aggression by another.

Mechanisms of Acquired Immunity.—There seem to be four ways in which *increased resistance* to a disease can manifest itself in an animal. (a) There may be some increase in the nonspecific factors of resistance, such as a decrease in the permeability of the skin and mucosa. (b) The tissues may be altered in such a way that they become intrinsically resistant. (c) Mobile cellular elements (phagocytes) may be made available more quickly to fight off the invading organisms. (d) Soluble substances which tend to protect against infection (antibodies) may be secreted into the blood and tissue fluids.

Immunity vs. Hypersensitivity.—The changes which occur in an organism in response to an infection may enable it to throw off the infection, and are beneficial in the sense that resistance to subsequent infections of the same type will be greater. The mechanism of these changes, however, is evidently so general that they can be provoked by influences other than infectious agents. Non-

infectious, dead microorganisms may cause such changes, and even apparently harmless substances may sometimes cause alterations in the reactions of the tissues or the substances in the circulation. An animal may become highly reactive (hypersensitive) to certain harmless substances. Burnet[18] has supposed that the basic mechanism of all reactions of an organism to foreign substances consists in the recognition by the tissues of the organism of the differences between "self" and "not-self." You do not become hypersensitive to any of your own blood proteins, and your stomach, although it will digest tripe, refuses (usually) to digest itself—so long as you are alive. The defenses which an organism puts up against infection do therefore have something in common with the processes of digestion, just as Metchnikoff thought.

"Pseudo-defense" measures, in the case of usually harmless substances such as ragweed pollen, may set up a mode of reaction which is deleterious to the organism and constitutes what we call allergy. We can only suppose that on the whole the benefits of possessing the immunity-sensitization mechanism outweigh the harm, or otherwise the device would not have survived during the long course of evolution.

ANTIBODIES

Animals exposed to infection produce in the blood and tissue fluids soluble substances which tend to prevent or cure infection. These are called *antibodies* (because they are bodies acting against introduced substances), but the term, as we have seen, is not restricted to substances with a protective function. The substances that call forth the production by the animal of antibodies are called antigens (because they cause animals to *generate* the antibodies). There is a certain element of circularity in these definitions, which we shall not bother to remove.

Specificity.—It is an old observation that, although recovery from a disease may give an individual a longer or shorter period of immunity to this disease, it does not protect him against other, unrelated, diseases. If antibodies are one of the main bases of immunity, we should expect that they would also show this selective action, and this is the case.

The specificity of serologic reactions, extraordinarily sharp, but still limited, has been well defined by Landsteiner[81] as "the disproportional action of a number of similar agents on a variety of related substrates." This definition seems the best that has been proposed, although it would also include many chemical reactions, as might be expected if serologic specificity were fundamentally chemical in nature, which we now believe.

To sum up what we now know about the specificity of antibodies, we may say: antibodies can be sharply specific, and can distinguish minute differences in chemical or spatial configuration. Differences which are slight from the chemical point of view tend to be slight serologically, and in some cases, especially with neutral antigenic determinants, antibodies fail to distinguish marked chemical differences. Antibodies clearly must contain spatial differences corresponding to the differences of the stereoisomeric substances that they can differentiate. In some cases the specificity of antibodies is of a relatively low order, and they may be specific for a part of the molecule only, or even for certain radicals, such as $-AsO_3H_2$ or $-SO_3H$.[81] Antibodies may be

directed toward more than one determinant in the molecule, and even those directed toward one determinant must vary to some extent around a main pattern. Antibodies will react with simple chemical compounds as well as with proteins and other complex antigens.

Nonhomogeneity.—The weight of evidence indicates that in general an antiserum against a given antigen contains a diversity of antibodies not all alike.[80, 88, 137, 79, 68, 69, 70, 59, 51, 27, 135, 136] We shall not discuss these differences further.

Chemical Nature.—Antibodies are serum proteins, with physical properties indicating that they belong in the "globulin" class. An antibody clearly must differ in some respect from the other globulins in the serum, since it bears the imprint of its characteristic specificity, not possessed by the other globulins, and this must reside in some chemical difference in at least part of the molecule. Nevertheless, in most cases no marked chemical or physical differences have been observed between antibodies and so-called "normal" globulins.

Molecular Weight.—The molecular weight of antibodies in the rabbit (and probably in man) seems to be the same as that of normal rabbit globulin, *i.e.*, about 160,000, or in some cases somewhat more. Some horse antibodies seem to have molecular weights of nearly a million. Davis and his co-workers[29] found that Wassermann antibody (syphilitic reagin) dissociated from the antibody-antigen precipitate showed two components, with sedimentation constants of 7 (about the usual molecular weight) and 19 (high), respectively. Other workers have reported a high molecular weight for this antibody.[82]

Electrophoretic Mobilities.—In the Tiselius apparatus, normal horse serum shows components with four different electrophoretic mobilities; in order of decreasing mobilities these are the albumin, and three globulin components designated as α, β and γ globulins.[131] These three globulins evidently differ in net charge per molecule at the alkaline pH used, but are believed all to have the same sedimentation constant, $S_{20} = ca.\ 7.0$; the heavy component ($S_{20} = 18$) probably migrates with the α globulin.[76] Rabbit serum shows four components of approximately the same mobilities as the components of horse serum.

The α and β globulins of horse serum, at any rate, seem actually to be complex, each group consisting of two components, designated as α_1 and α_2, and β_1 and β_2, respectively.

In practice, comparison of electrophoretic patterns and of the areas under the respective peaks, rather than determination of actual electrophoretic rates, usually proves sufficiently informative.

It appears that horse anti-diphtheria toxin can have the characteristics of either a β (β_2) or a γ globulin. These two kinds of antitoxin show definite differences in serologic behavior.[75, 76]

Rabbit and human antibody seem generally to have an electrophoretic mobility corresponding to that of the γ globulins.

Seibert and Nelson[126] found that an antibody to tuberculin protein in rabbits had about the electrophoretic rate of an α globulin. Davis[29] found that Wassermann antibody (syphilitic reagin) dissociated from precipitates formed with the antigen had a mobility between that of the β and γ globulins.

Chemical Analyses.—There have been a number of analyses for nitrogen, amido-nitrogen, mono-amino, and di-amino nitrogen, and amino acids, in anti-

bodies and in specific precipitates containing a nonnitrogenous antigen. The chief results are summarized in Marrack.[96] On the whole, the differences between samples, and between antibody and normal globulin do not seem to be greater than could be accounted for by the use of different methods by different workers. Antibodies and normal γ globulins have about the same chemical composition. This indicates, within the accuracy of these determinations, either that the chemical differences between antibodies and normal globulins are slight, or that they depend chiefly on arrangement of the groups within the molecules.[108]

FORMATION OF ANTIBODIES

There has long been a large amount of evidence,[cf. 132] not always too consistent, which indicated that the site of formation of antibodies might be the reticulo-endothelial system, or at least that the antigenic material was taken up by these cells, and perhaps put through a preliminary processing there.

Considerable evidence[40, 34, 33, 52, 35, 41, 75] has accumulated that lymphocytes also form antibodies. Ehrich and Harris[41] point out that we do not know the exact role of granulocytes and the macrophages, aside from that of dissolving particulate antigens (the only function of these cells which has actually been observed), and aside from their retention of antigenic material which would otherwise be excreted or destroyed elsewhere in the body. The importance of the role of the reticulo-endothelial system may or may not be diminished by these new findings.[74, 42]

The Anamnestic Reaction.—When a specific antibody has once been produced by an animal as a result of contact of the tissues with a foreign protein, after contact ceases the amount of circulating antibody gradually decreases with the passing of time until, at length, none at all can be detected. If, now, the antigen enters the tissues again, the specific antibody will appear in the circulation, and will reach a given level in a much shorter time than was required following the first contact.[118]

Mechanism of Formation.—The "side-chain" theory of Ehrlich supposed that antibodies were specific chemical combining groups or receptors of the cells, always present on the cells, but produced in increased numbers and cast off into the circulation following an antigenic stimulus. It now has hardly more than historical interest. It carried with it the necessity of supposing an enormous number of different preformed receptors, which alone is sufficient objection. Another early hypothesis, revived some years ago, that the injected antigen, or part of it, was in some way incorporated in the resulting antibody, has been disproved.[31, 7, 62, 65, 66, 67, 141, 59]

Some have supposed that the antigen is digested preparatory to the process of antibody formation. This is improbable, since digestion would tend to destroy the characteristic structures in which specificity resides. Also, antibodies can be obtained to chemical groups which the animal body cannot digest. A further objection comes from Landsteiner's[81] tests on antibodies specific for fatty acids.

The first theory of antibody formation proposed which still seems at all probable today is that put forward independently by Breinl and Haurowitz,[14] Alexander,[3] and Mudd,[102, 65] Its chief distinguishing feature is that it assumes

that antibodies are new globulins, synthesized under the directing influence of a molecule or particle of antigen which is there.

It is supposed that as a new molecule of globulin is formed in the cell, the arrangement of the chemical groups on part of its surface is partly determined by the fact that a molecule, or part of a molecule of antigen, is adjacent to it. It could be imagined that the presence of a strong polar group, for example, on the surface of the antigenic particle, might prevent the establishment of a polar group of like charge immediately next to it in the antibody, which is being constructed, and might lead to the laying down of a group of opposite charge. In this way a configuration might result on the surface of the antibody which reflected, "in reverse," certain distinctive characteristics of part of the surface of the antigen molecule. It is necessary to suppose further, either that the molecule of antibody, when completed, is cast out of the cell, and a new molecule then formed under the influence of the persisting molecule of antigen, or that the molecule of antibody, once formed, can bring about the formation of other molecules like it, which is perhaps less likely, although it may have some analogies, as in enzyme formation, for example. The chief objection to this theory would seem to be the fact that there is hardly any conceivable way to prove it. However, it must be admitted that the available evidence [cf. 121, 122, 55] accords well with it. More recently Pauling[108] proposed a somewhat modified form of this theory.

Burnet[19] does not think there is any adequate reason for believing that simple juxtaposition of the growing antibody molecule to the antigen would result in a complementary pattern. Instead, he thinks it more likely that certain proteinases (or other enzymes also?), in the antibody-forming cells are lastingly modified as they are engaged in destroying the antigenic particles. Then these "trained" enzymes synthesize the antibodies. This hypothesis has the merit of a certain plausibility; data to prove it are still lacking, as is true also of older theories.

Pauling and Campbell[110] have claimed to be able to confer antibody-like properties on globulins and other proteins by slowly reversing (in vitro) the denaturation of denatured protein while it is mixed with an antigen or hapten. They supposed that during denaturation the peptide chain unfolds, and when denaturation is reversed it refolds in such a way as to assume a configuration complementary to that of the antigen. They therefore interpret their results as confirming the theory of antibody formation propounded by Pauling. This work received considerable publicity, although the claim to have made antibodies in vitro is not new,[39, 120, 82] and a dramatized version of it was broadcast on the radio. Nevertheless, it does not seem at the present time that any outstanding immunologist believes that real antibody has ever been made artificially.[74]

Measurement of Concentration.—In the early stages of immunology, when the chemical nature of antibodies was completely unknown, methods of determining them quantitatively could only be comparative, and even these were very crude. The essence of practically all these methods was to make successive dilutions of the serum and test for the antibody activity, recording the highest dilution which was still active.

Immunologists were baffled for some time by the problem of estimating the actual amount (weight) of antibody in an antigenic serum, as no method was known to distinguish the antibody chemically from the other constituents of the serum. Rough determinations were made by Landsteiner and Prasek.[87]

The use of an antigen containing some special chemical characteristic by which it could be differentiated from the antibody offered a way out of this difficulty.[142, 143, 59] It then became possible to analyse the precipitate for nitrogen, thus finding an estimate of the total protein present, then to determine independently the amount of antigen, and by subtraction, the amount of precipitating antibody. If the precipitate were made in such a way as to obtain all the detectable antibody in the serum, one could thus estimate the actual weight of antibody protein per volume of serum. Heidelberger and Kabat[58] later showed that if suitable blanks were done on the bacteria, it was also possible to determine by nitrogen analyses alone the amount of *agglutinating* antibody in a serum.

ANTIGENS

Conditions of Antigenicity.—The majority of antigenic substances are proteins, and it was formerly believed that only proteins could act as antigens. It is now known that some lipoids, particularly cholesterol and related compounds,[82] when injected and mixed with antigenic protein, produce antibodies. Purified specific capsular polysaccharides of the pneumococcus are devoid of antigenicity for rabbits and horses, but they can, under proper conditions, call forth the production of specific antibodies in a mouse or a man.[36] Lipoid-carbohydrate compounds, even when carefully purified to remove protein, will produce antibodies when injected.[43, 5, 44, 8] Certain substances belong to this latter class are hardly inferior to proteins as antibody producers.

As a rule, an animal's own proteins, or those of another individual of the animal's own species, are not antigenic for it. In immunology this principle has received the name of *horror autotoxicus* ("fear of poisoning oneself"), which is thus stated: an animal will never produce antibodies to any substance naturally found in its own circulation.

The principle as stated above is qualified by the restriction that it applies only to substances in the animal's circulation. This qualification is needed, for it has been found that protein from the lens of the eye[135] will produce antibodies in animals of the same species,[78, 95] and it has been claimed that a guinea pig can be sensitized with the lens of one eye and later shocked anaphylactically with the lens of the other. Lewis[91] found that lactating goats could be caused to form antibodies to their own casein. These antibodies reacted to an equal degree with cow casein. Hektoen[91] reported obtaining other *iso-antibodies* in rabbits, against thyroglobulin, the iodine-containing protein of the thyroid gland; and fibrinogen from the blood, although Stokinger and Heldelberger[129] later found definite differences in their antisera to thyroglobulin from man, hog, cow, or sheep. Thus, it seems established that certain proteins of the body, which never normally come into the circulation, can act as *iso-antigens* (antigens for individuals of the same species as the individual producing them. Lewis[92] found that the tissue lipoid extracted by alcohol from the brain was iso-antigenic when injected mixed with foreign serum.

The specific determinants in natural proteins are combinations and arrangements of amino-acid residues. Though carbohydrates may be present, or metal-containing prosthetic groups as in hemoglobin, no case has thus far been studied in which the specificity was determined by them.

Number of Reactive Groups (Valence) in an Antigen Molecule.—Since we do not know the exact nature of the determinants in natural proteins, we cannot determine accurately how many antigenic determinants are present in a single molecule. From analyses of precipitates for antibody and antigen, however, it is possible to determine the maximal number of antibody molecules which can combine with a molecule of antigen; the valence of the antigen cannot be less than this. We thus set a lower limit for the valence of the antigen molecule. The minimal values obtained (references in[71]) are given in Table I.

TABLE I

ANTIGEN	VALENCE	MOLECULAR WEIGHT
Ovalbumin	5	40,500
Viviparus hemocyanin	231	6,630,000

Effect of Chemical Alteration and Conjugation on Specificity.—Almost any decided chemical change in a protein alters its specificity. The extensive and careful studies of Landsteiner[82] and his followers on chemically altered antigens have done much to throw light on the chemical mechanisms underlying immunity. Landsteiner's conclusions are best stated in his own words.

Conjugation.—"First of all, the nature of the acid groups is of decisive influence. Sulfonic acid immune sera reacted markedly with several sulfonic acids, but little, if any, with carboxylic acid antigens, and immune sera to the latter only exceptionally gave distinct reactions with azoproteins containing sulfonic acid groups. The determining influence of the arsenic acid radicals was still more pronounced, as is indicated by the fact that arsanilic acid serum precipitated all of the six substances tested which contain the group AsO_3H_2, and none of the other antigens."

Another important influence of acid groups is that their presence seems to define more sharply the specificity of the nucleus in which they are found, so that other substituted groups in the ring exert more influence on the specificity than they do in neutral antigens.

Haurowitz[54] has shown that strongly basic groups are probably just as effective as acid groups in directing specificity.

Landsteiner continues: "Another regularity, as seen from the very specific reactions of the three isomeric aminobenzoic acids and aminocinnamic acids, is that the relative position of the acid radical to the azo-group (the point of attachment to the protein) determined the specificity and appearance of cross reactions."

It was of the greatest interest to investigate the specificity of peptides (compounds made up of amino-acids), since proteins are made up of peptide chains. Landsteiner and van der Scheer carried out this investigation in analogous fashion.

It was found that the antisera to these compounds were quite specific, so that strong cross reactions were obtained only with closely related amino-acids, glycine and alanine, valine and leucine, and aspartic and glutamic acid, which, however, could be differentiated without difficulty.

Study of synthetic artificial antigens has shown that antibodies produced by them may be of several sorts. In the first place, especially if the chemical treatment has not been too drastic, the serum of an animal immunized with such a conjugated protein may contain antibodies directed towards the protein substrate, having no relation to the new specificity. It may contain antibodies directed chiefly towards the introduced groups, or these groups and the parts of the protein molecule with which they combine. Also, antibodies may occasionally be found whose combining group is directed simultaneously towards the original protein and the introduced group.[60, 53, 99]

The weight of evidence indicates that in general an antiserum against a given antigen contains a diversity of antibodies not all alike.[80, 88, 137, 79, 68-70, 58, 51, 27, 50, 135, 136]

In some instances these antibodies are distinct, that is, some are directed toward one part of the molecule, and some toward another. This has proved fairly easy to establish in the case of antibodies to conjugated antigens, but in the case of natural proteins, although it has been suggested[69, 27] it would seem that it has not yet been actually proved.[90] It has also been established in at least one case for the part of the antibodies directed towards the introduced group, that is, antibodies specific for different parts of the haptenic compound have been detected,[89] and may be present in other instances.[68, 6, 100, 101] There is hardly any doubt that if a sufficiently large, complicated molecule containing acid groups, were used as the hapten, distinct antibodies could almost regularly be obtained.

Influence of Optical Activity.—The differences observed in antibodies to aromatic compounds containing the same substituents, but in different positions, indicate that the spatial arrangement of groups, as well as their chemical nature, is of importance in immunology. This is in line with what was already known of other aspects of biological chemistry, where spatial arrangement has been found important.

Landsteiner and van der Scheer[82] differentiated serologically between d- and l-para-aminobenzoyl phenylamino acetic acids. For a second experiment, the three forms of tartaric acid, dextro, levo, and meso, were used, as usually linked to the protein through an amino group. The antisera differentiated all three of these isomers.

Studies by Goebel and Avery[49] later carried the differentiation of steric isomers still farther.

It is known that the specific capsular polysaccharides of pneumococci of types 2, 3, and 8, are constituted from molecules of glucose and glucuronic acid.[46] In view of the prominent effect of acid groups, we might expect the specificity of antibodies to these polysaccharides to be directed chiefly towards the glucuronic acid. This seems to be the case.

In later work with cellobiuronic acid (a disaccharide composed of one molecule of glucuronic acid and one of glucose), which seems to be the struc-

tural unit of type 3 pneumococcal polysaccharide, Goebel[47] obtained antisera that would precipitate type 3 polysaccharide when this was combined with a heterologous protein. Antigens containing cellobiuronic acid reacted vigorously with antipneumococcus sera of types 2, 3, and 8. As would have been expected, the antiserum to antigen containing the cellobiuronic acid conferred passive protection on mice to infection with virulent pneumococci of types 2, 3, and 8.[48] This is apparently the first instance of the production of effective immunity to an actual disease by the injection of an artificial antigen containing a purely synthetic hapten.

Haptens.—The term hapten was introduced by Landsteiner, and is thus defined by him: ". . . specific protein-free substances, although reactive in vitro, induced no, or only slight antibody response. For serologically active substances of this sort, in contradistinction to the protein antigens which possess both properties, the term hapten has been proposed. . . ."

It will be seen that this definition includes most of the carbohydrates, the Forssman "antigen," and the large numbers of simple substances which have been found to react specifically with appropriate antisera.

Inhibition Reaction.—Although antisera against conjugated antigens containing simple chemical compounds were often found to be quite specific for the particular hapten used, and readily precipitated antigens containing the hapten coupled to another protein, nevertheless Landsteiner observed that no precipitation or other visible reaction occurred if the antisera were simply mixed with the hapten alone, instead of with the hapten-protein compound. This did not at the time seem surprising, since it was thought that only substances of high molecular weight, which gave colloidal solutions, could take part in the precipitin reaction. Later observations cast some doubt on this idea.

Landsteiner, reasoning that nevertheless some reaction must occur, since the point of union in the case of the artificial antigens was obviously the hapten, took account of the fact that precipitation by immune sera is prevented or diminished when an excess of the antigen is present, and deduced that the addition of an excess of the *hapten* might prevent the precipitation by antibody of the artificial antigen containing the hapten. Experiments proved that this actually happened, and specific inhibition of the precipitin reaction was obtained by the addition of the simple chemical compound towards which the antibody was directed, or by related compounds. The chief new result was to show that antibodies could react, not only with protein, but—unexpectedly—even with very simple chemical compounds, such as benzoic acid or tartaric acid. This technique has been of great value in studying the immunologic reactions of drugs, etc.

Species Specificity.—We observe that the specificity of proteins is usually different when they are from different species, even when the proteins are functionally analogous. The more closely related any two species are, apparently, the greater the serologic likeness of their corresponding proteins.

There can be no doubt that these serologic similarities and differences in proteins from different species are due to chemical similarities and differences in the proteins themselves. In other words, as two species diverge in the course of evolution, chemical as well as morphologic differences develop, and

proteins fulfilling a given function in two different species no longer have quite the same chemical composition. This is reflected in their immunologic characteristics.

The most extensive investigation of species specificity was the classical work of Nuttall[105] who studied the serum proteins of a very large number of species, testing them with antisera from rabbits.

Toxins.—Toxins are antigenic poisons.[26] Because of their clinical importance, they are intensively studied in the early days of immunology, and it is still customary in many books to illustrate immunological phenomena by reference to toxins and antitoxins, although we know now more about the composition of certain other antigens.

Certain toxins have been purified chemically,[38, 106] and even crystallized.[128, 82, 111]

Lipoid Antigens.—The question of the possible antigenicity of lipoids has long been debated. It has not been demonstrated that any pure lipoid is by itself capable of inciting antibody formation, but antibodies have been obtained by injecting lipoids mixed with protein. In particular, lecithin (even synthetic) and cholesterol have been found antigenic in this sense by some workers, but others have reported failure when purified lecithin preparations were used.

Boivin and Mesrobeanu[9, 8] discovered a new type of antigen. By extracting *Salmonella typhimurium* with 0.25 trichloracetic acid in the cold, they obtained a complex which consists of a nonantigenic specific polysaccharide, acetic acid, fatty acids, and phosphoric acid. This substance was not dialysable, gave opalescent solutions, was toxic, specific, and antigenic. On hydrolysis, fatty acids and the specific polysaccharide were liberated. Neither of these is toxic or antigenic. Similar results were reported by Raistrick and Topley,[117] and such complexes have been isolated also from dysentery bacteria, various species of Pasteurella, *Past. aeruginosa, B. anthracis, Eberthella typhosa, Proteus* X_{19}, etc.[24]

Bacterial Types.—Bacteria as a group are probably a heterogeneous group of more or less unrelated forms,[36] classified together because of their being roughly the same size. Naturally, therefore, the antigens contained in them may vary widely.

The study of chemical bases for bacterial types still has a long way to go, in spite of the notable results achieved in the study of pneumococcal types[16, 47, 96] and other bacterial forms. A knowledge of the chemical composition of the various micro-organisms will be of especial importance for the allergist. In this connection, the work of Seibert[125, 127] and others on tuberculin is of interest.

Bacteria have long been classified into Gram-positive (retentive of a basic dye) and Gram-negative (not retentive of basic dye) forms. Dubos[36] believes that the Gram-positive character may reside in a protein-ribonucleate complex which exists at the bacterial cell surface.

ANTIBODY-ANTIGEN REACTIONS

The majority of those actively engaged in studying immunologic reactions are convinced the reaction between antibody and antigen is chemical in nature.

The reaction between antibody and antigen, and that between proteins and proteolytic enzymes are the only instances which have been well studied of reactions occurring between molecules of very large size; it need not be surprising if some new features emerge. There are several features which stand out, and which we may present in summarized form.

1. *The Reaction Is Specific.*—This point has already been extensively discussed, and it will not be necessary to give any examples here. We shall have to keep in mind the specificity of the reaction when we come to discuss the mechanism of union, that is, the nature of the forces holding antibody and antigen together.

2. The *entire* molecules react, not some fragment split off from them. This is clearly shown by ultracentrifugal experiments.[63, 107]

3. The antibody molecule, and molecules of protein antigens, at any rate, behave in these reactions as fairly *rigid* ellipsoids of greater or less eccentricity. This is obvious if we accept the views of Neurath[103] on the shape of protein molecules. Boyd and Hooker[11, 12] found that the composition of antibody-antigen precipitates is compatible with the idea that antigen molecules are roughly spherical, and antibody molecules behave as elongated units, such as ellipsoids, or chains of connected spheres. It is also found in antibody-antigen reactions between films of the reagents[115] that the thickness (30 to 50 Å) of the films is such as to suggest that the molecules are behaving like ellipsoids lying on their sides.

4. No *splitting, digestion,* or other profound chemical alteration takes place in the reacting molecules after they have combined.

5. The combination of antibody and antigen takes place at the *surface* of the molecules. This follows from the third point discussed above.

6. The union between antibody and antigen is a *firm* one, but it is at least partially *reversible.*

7. *Both antibody and antigen enter into the specific precipitate,* or into the specific "agglutinate."

8. *Antibody and antigen can combine in varying proportions.*

9. Serologic reactions display certain analogies with *colloidal* reactions.

The Two Stages of Serologic Reactions.—It is customary to consider serologic reactions as occurring in two stages. Such a subdivision should not be taken as necessarily implying that the two stages do not overlap, for it is quite likely that the second stage sets in before the first is entirely completed.

These two stages are: (1) the specific combination between specific groups of the antibody and corresponding groups of the antigen or hapten; (2) the secondary, observable reactions which may follow this, such as precipitation, agglutination, complement fixation, etc.

First Stage: Combination.—For a long time the principal chemical question in this connection was: is the union solely due to Coulomb forces (attraction between positive and negative charges, e.g., $-NH_3^+$ and $-COO^-$) or are there other forces involved?

It is, however, likely that other forces, such as those classified as secondary valence, play a role in serologic reactions.[111, 109] It does not seem likely that the covalent bond is concerned.

Size of Reacting Groups.—The size of the specific determinants in natural proteins is still not exactly known, but in the case of artificial antigens we can form some idea of the extent of the reacting group.

The total reacting group in conjugated antigens is probably somewhat larger than the introduced group which chiefly determines the specificity because of some influence contributed by the adjacent groups of the native protein. This means that the size of the reactive groups is to be thought of as of the order of ordinary synthetic organic molecules.

Rate of Combination.—When precipitin and antigen are mixed, the precipitate often appears instantaneously, so far as can be observed. Heidelberger, Treffers, and Mayer[64] found that combination between antibody and egg albumin, even at 0° C., was apparently complete in twenty seconds. Eagle[37] states that in the flocculation test for syphilis the combination between the reagin and the lipoid "antigen" is more than 95 per cent complete within one minute after their mixture.

Second Stage: Neutralization, Complement Fixation, Precipitation, etc. (a) *Neutralizations of Toxins and Viruses.*—Since the flocculation of toxins by antitoxins was not at first known (being missed because of the narrow zone in which precipitation is obtained), and because the point of primary clinical interest is the toxicity of the mixture, most of the studies on toxin-antitoxin reactions have been made by mixing toxin and antitoxin in varying proportions and testing the toxicity on animals. It is probable that in such reactions we have an opportunity to study the characteristics of the primary stage of serologic reactions, practically independently of the complications introduced by any second stage.

For the nature of the tests made, it was natural to study the relation of the amount of bound toxin to the amount remaining free.

Even knowing only approximately the numerical laws governing toxin-antitoxin reactions, we can now see why the early workers were often puzzled by the quantitative relations they observed. The addition of 0.2 ml. of antilysin to a certain amount of tetanolysin lowers the toxicity to about 35 per cent of its initial value. The addition of another 0.2 ml. of antilysin has a much smaller relative effect, for the toxicity is still nearly 15 per cent of the initial value. In a typical experiment, we find that the addition of 0.1 ml. of antiserum has neutralized 30 per cent of the toxin. It might have been expected that the addition of three times as much (0.3 ml.) would neutralize 90 per cent, but actually it neutralizes only 78 per cent, and the addition of 1.0 ml., which we might have expected to neutralize three times as much toxin as was used altogether, actually neutralizes only 96 per cent of that present, leaving 4 per cent still free.

(b) *The Precipitin Reaction.*—The actual amount of precipitate obtained from a given amount of antiserum depends in the first place on the amount of precipitating antibody present, and in the second place on the amount of antigen added. If too much antigen is added to the mixture, no precipitate is formed ("inhibition zone"). If too much of certain sorts (especially horse) of antibody is added, no precipitate is formed ("antibody inhibition zone").

With others (most rabbit antibodies) no inhibition results if antibody is added in excess.

Mechanism of the Precipitin Reaction.—There have been several hypotheses proposed to account for the second stage of serologic reactions where precipitation or flocculation is produced. As yet, there is not general agreement as to the correctness of any particular one of these, so it will be desirable to say something of each.

Theories of Arrhenius.—Anxious to apply the law of mass action to immunologic reactions, Arrhenius tried to show that all the quantitative data then known could be accounted for on the basis of reactions obeying the laws of classical chemistry, without making use of the concepts of Ehrlich, which involved the assumption of the existence in the same system of a multiplicity of antibodies and antigens of different characteristics. The figures of Arrhenius show good numerical agreement with the experimental data, but his very success is now a lesson in caution, demonstrating again that numerical agreement between experiment and the predictions of a theory does not always show that the theory is correct. Since the composition of the antigen-antibody compound could not then be determined, and nothing was known of the molecular sizes of antibodies, antigens, or their reaction products, Arrhenius was able to make any assumptions he liked about the relative numbers of molecules taking part in the reactions, and the numbers of molecules of product formed. In the light of modern knowledge, most of these assumptions are seen to be in error. Thus, to account for the results of an experiment on the neutralization of ricin by antiricin, Arrhenius assumed that two molecules of antiricin reacted with two molecules of ricin, to produce three molecules of innocuous substance. At that time, little was known of the composition of the products of serologic reactions, but today it is evident that this is an extremely improbable reaction.

Furthermore, in most cases, as in the antiricin-ricin reaction, Arrhenius did not consider the possibility that the reactants could combine in multiple proportions, although we now know this to be one of the outstanding characteristics of serologic reactions.

In an attempt to account quantitatively for the inhibition zone of the precipitin reaction, Arrhenius did make the assumption that the reagents could combine in two different proportions, one of them giving a soluble compound. On this basis he was able to predict the *amounts* of precipitate observed relatively satisfactorily. From what we now know about the composition of the precipitate, however, it is clear that any predictions he might have made as to the composition of the precipitate would have been wrong.

Theories of Bordet.—Perhaps the chief credit for insisting on the power of antibodies and antigens to combine in varying proportions goes to Bordet,[10] although others had proposed the idea previously.[57] Bordet stressed the similarity of serologic to "adsorption" reactions, and went so far as to deny the applicability of the laws of ordinary chemical union to serologic reactions.

We now know that there is no such fundamental distinction between adsorption and ordinary chemical reactions as Bordet thought. Practically any reversible reaction taking place at a surface where there are many reactive

groups will follow a course similar to that of adsorption reactions.[45] Consequently, this difference between the views of Bordet and of Ehrlich or Arrhenius has reduced simply to the insistence of the former on combination in multiple proportions.

The "Alternation"—"Lattice"—"Mutual Multivalence"—"Framework" Theory.—Marrack[96] pointed out that if the antibody has more than one combining group, it might be possible for antibody and antigen to be bound together in the form of a coarse "lattice." This involves a new concept, in that the aggregation is ascribed not to a loss of attraction of water, as in Bordet's theory, but to specific attraction between the particles. "This mutual attraction is due to the link provided by further antigen molecules."[96]

A very similar theory was developed by Heidelberger and Kendall[57] and later taken up by Pauling,[108] and various other workers.

(c) *Anaphylaxis.*—Portier and Richet[116] reported that toxic extracts of the tentacles of certain sea anemones, in doses so small that they produced no symptoms in normal animals, would, if injected into a dog which had recovered from a previous—sublethal—dose, cause violent illness, and often death. To characterize the *lowering* of resistance thus evidenced, they coined the phrase, anaphylactic action ("action anaphylactique de certains venins"), thus calling attention to its antithesis to the prophylactic or protective effects following other forms of treatment.

Other workers (Magendie, Flexner, Richet, and Héricourt) had previously observed what was doubtless anaphylaxis, and Arthus, Theobald Smith, and Rosenau and Anderson subsequently noticed similar phenomena. It was discovered that the substance provoking anaphylaxis need not be toxic in itself; on the contrary, normal animal serum from a different species, or harmless substances such as egg white, would produce the effect. It was only necessary to "sensitize" the animal by one or more previous injections, and then, after an appropriate interval, administer the anaphylaxis-provoking, or "shocking," dose.

Mechanism of Anaphylaxis. Antibodies.—There can hardly be any doubt that the reaction of anaphylaxis is essentially the result of an antibody-antigen reaction. Evidence that antibodies play an essential role is overwhelming, and hardly needs to be summarized here.

Cause of the Symptoms.—There have been two theories. One supposed that union of the antibody with the antigen disturbed the "colloidal equilibrium" of plasma or cell protoplasm, so interfering with the normal physiology of the tissues that shock is introduced (physical theory). The other theory supposed that as a result of the antibody-antigen combination a toxic substance was produced which caused the shock (chemical theory). If we are willing to grant that the toxic substance does not have to come from the antibody or antigen, but from the tissues themselves, it would seem that the chemical theory is better supported by the evidence,[132, 124] and it seems decidedly less vague.

It has been suggested[1, 93] that the toxic substance responsible for anaphylactic shock is histamine, or a histamine-like substance. Lewis[93] found that

the local reddening and succeeding local wheal, following a local stimulus or injury to the skin, were independent of the local neuro-vascular mechanism. He concluded that they were the result of a chemical stimulus from some new substance coming from the injured cells; and he found that of the many substances he tested, only histamine would produce the same effects.

Some observations which seem contrary to the histamine hypothesis have been cited, such as differences in the response of rat uterus to histamine and behavior during anaphylaxis, although according to some workers, the two reactions in vivo are essentially the same. Campbell and Nicoll[21] found a substance to be released by guinea pig lung during anaphylactic shock which stimulated rat uterus, which is unaffected by ordinary doses of histamine. Went[138] considers the most likely hypothesis is that during anaphylaxis, different biologically active substances are released from different tissues, depending on the chemical nature of the tissue. For example, histamine might be liberated from the lung, choline from the heart, adrenalin from the adrenals, etc. Went obtained evidence that the isolated guinea pig heart behaves during anaphylaxis as if stimulated by a substance of the choline group, rather than histamine. He also believes that the pathologic changes of anaphylaxis occur not only because of the increased liberation of histamine, choline, etc., but also in part because the shock organs of the sensitized animal are more susceptible to these substances.

It is worth noting that a number of workers have found the enzyme histaminase does not protect against anaphylaxis.[2, 119]

(d) *Hypersensitiveness in Man. Serum Sickness. Mechanism.*—An explanation of serum sickness which still seems the most plausible was proposed by von Pirquet and Schick.[113] Their suggestion may be paraphrased thus: following the injection of foreign serum, the patient begins to form antibodies against certain of the serum proteins. After a time, these antibodies appear in amounts sufficient to react with the antigens responsible for their production, traces of which have persisted in the patient's circulation. This reaction is supposed to cause the symptoms. The mechanism would thus be quite similar to that of anaphylaxis, except that the latter is never observed to follow the primary injection.

(e) *Allergy.*—Von Pirquet coined the word "allergy," meaning literally "changed or altered reactivity" to denote any acquired specific alteration in the capacity to react, which occurs in living organisms or tissues upon exposure to living agents or inanimate substances. As originally framed, this definition was meant to include alterations in the direction of protection as well as in the direction of hypersensitivity, so that, strictly, immunity would also be connoted by the word. Von Pirquet felt that a single word was desirable to embrace all these different specific alterations in reactivity, since he felt, as we do, that they were all fundamentally related biologic phenomena. However, it has not proved convenient to use, in practice, a word which is, as it were, two-edged, so that if an animal is said to be allergic to an agent it is not known whether he is more or less resistant. The word allergy, therefore, although never authoritatively redefined, has in actual usage lost the

connotation of immunity, and now implies what we have above expressed as hypersensitiveness, except that it is not usually used to denote anaphylaxis.

The chemistry of allergy has been reviewed by Newell[104] and by Coulson and his associates.[28] The allergen in pollen is reported to have a molecular weight of about 5,000.

It is now believed that a hereditary factor[25, 133, 139] is often involved in allergy, but, of course, contact with the allergen[25] is also necessary.

Allergy to simple compounds (drug allergy) indicates that the incitant need not necessarily be a complex antigen.

In some cases the patient's reaction is caused, not by the whole drug molecule, but by a portion of it, or some chemical group contained in it. In allergy to arsphenamine it has been found that some patients are sensitive to the organic part of the molecule, while others react to the arsenic. It has been claimed that iodoform sensitivity may be directed toward either the methyl radical or the iodine. It was found by Dawson, Sanders, and Tomlinson[30] that individuals allergic to quinine could be separated into several groups, depending on which part of the molecule was responsible for the sensitivity.

An interesting feature of drug allergy is that nearly all of the incitants are substances which are not antigenic. If we are to suppose that the fundamental mechanism is the same as in the forms of allergy already discussed, the problem arises, how can a nonantigenic agent sensitize? Following the publication of the work of Landsteiner and his co-workers, which showed that it was possible to render simple chemical compounds antigenic (haptenic) by attaching them chemically to proteins, it was apparent that a possible clue to this mystery had been found. It might be that allergenic drugs become so only by combining with the susceptible individual's own proteins. This suggestion had, in fact, been previously made, but without sufficient evidence.

There is some reason to think that drugs may be able to attach themselves to proteins in the body. In some cases it is easy for a chemist to see how this might occur; in others it must be supposed that the attachment takes place after chemical alteration of the drug in the body. In fact Mayer,[98] studying sensitivity to azo dyes and aniline derivatives, found various group reactions, based not on the complete original substances of exposure, but to new derivatives formed in the body by oxidation, reduction, etc. An argument in favor of the formation of conjugates comes from the work of Landsteiner and Jacobs,[86] who studied the chlor- and nitro- substitution products of benzene. Treatment with sodium ethylate or sodium methylate was found to remove a chlorine atom or a nitro group much more readily from those substances which would sensitize guinea pig skin than from the nonsensitizers. It was also found that the sensitizing substances alone gave stable substitution compounds with aniline. More recently, Landsteiner and Chase[83, 84] have succeeded in sensitizing the skin of guinea pigs to a simple compound (picryl chloride) by injections of a conjugate of this substance with guinea pig stromas.

Although this seems to be a possible mechanism for the sensitization to drugs, it will, of course, be realized that its operation has not actually been demonstrated in the majority of cases, and the situation is such that the dem-

onstration would in many instances be very hard. In addition, there remains a difficulty. So far, no one has demonstrated the presence of any sort of antibodies in drug allergy, except anaphylactic antibodies after treatment with picryl chloride.[83] This does not, of course, prove that antibodies are not present, as our methods of testing for them may be inefficient, or they may be almost completely confined to the tissues (sessile). Even skin tests, except in eczematous drug eruptions, are as a rule of little or no value in determining the excitant in drug allergy.[130]

In experimental allergy to simple compounds, satisfactory sensitization is usually produced only by treatment of the skin, except under special conditions.

There are some observations which suggest that sensitization to simple compounds is more likely to result when they are applied to an injured area, as the sensitization to picric acid which occasionally results from the use of butesin picrate on burns. Also, Landsteiner and DiSomma[85] found that picric acid, which alone did not sensitize guinea pig skin, would do so when painted on after the application of cantharidin.

Atopy.—The term atopy, meaning "a strange disease," was proposed by Coca to denote certain clinical forms of human hypersensitiveness which are affected by a hereditary predisposition. It was once supposed that this condition did not occur in animals, but since that time it has been observed in dogs,[20, 114, 134] cattle,[13] and other animals,[72, 17, 140, 23] including a walrus.[123]

Mechanism.—Just as there is good reason to think that anaphylaxis is an antibody-antigen reaction, so there is persuasive evidence that the reaction in the natural or atopic type of allergy is also a reaction between the allergen and an antibody, which is in this case called reagin. We may cite two main facts: (1) individuals with atopic types of hypersensitiveness such as hay fever, asthma, and eczema, have reagins in their blood. These reagins are specific for the allergen and their concentration usually parallels the degree of skin sensitivity. (2) The reagin content of the blood is observed to increase to from two to four times its initial level following the injection of pollen extracts, suggesting that the reagins are produced in response to the antigenic stimulus of the allergen.

ALLERGY OF INFECTION

Allergy in Tuberculosis.—Koch[77] made the basic observation. The distinguishing characteristics of the tuberculin type of reaction are: (a) there is an incubation period of several hours before the reaction shows itself; (b) the reaction is inflammatory in nature; (c) hyperpyrexia is characteristic of the general reactions; (d) the symptoms are very similar in different species, and are different from those of anaphylactic shock.

Thus bacterial disease agents seem to act in one or both of two ways, i.e., to lead to the formation of protective antibodies, or to produce in the host hypersensitiveness or allergy to some constituents of the bacterial cell. The last action itself may be of two kinds, depending on whether there is produced an anaphylactic type of sensitiveness, similar in nearly every way to that

produced by ordinary nonbacterial antigens, or the tuberculin-type of sensitiveness. The response to bacterial agents may be summarized schematically:

1. Production of protective antibodies
2. Production of allergy
   (a) anaphylactic allergy
   (b) tuberculin type allergy (allergy of infection)

The immunochemical reason for the difference between the tuberculin and other types of hypersensitiveness is not understood. Some workers are convinced that bacterial allergy is on the whole not so very different from other forms of hypersensitiveness. The reader may find a good presentation of this point of view in a paper by Bronfenbrenner.[15] It is a fact that tuberculin allergy is one of the more extreme examples of bacterial allergy.

Relation of Tuberculin Allergy to Antibodies.—Rich[118] maintains that there are, nevertheless, a number of persuasive reasons for believing that allergy of infection depends upon specific antibody. Among the points he mentions are: (a) the fact that antibody cannot be demonstrated in the circulation might simply mean that a very small amount is sufficient to produce the allergic state; (b) the reaction shows a high degree of specificity, making it difficult to account for it on any other basis; (c) the allergy of infection exhibits the anamnestic reaction as does antibody production; (d) the sensitivity can be depressed or abolished by specific desensitization.

SUMMARY

A review of recent developments in the field of immunity, from the point of view of a chemist, but with special reference to the interests of the allergist, is given. A consistent—and therefore possibly oversimplified—interpretation is adopted, which treats of all immunologic phenomena as the result of the interaction of antibodies (specifically modified serum globulins) with antigens or haptens (small molecules capable of reacting with antibodies).

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American Academy of Allergy

NEWS AND NOTES

REPORT OF THE COMMITTEE ON PHARMACEUTICALS AND MEDICAMENTS

The report on pyribenzamine and anthallin was approved.

The formulas of Dr. Gay of Biloxi are to be sent to the Secretary of the Council of Pharmacy of the American Medical Association for consideration and comment.

In answer to a query concerning the meaning of the phrase, "Stamp of Approval" appearing in the by-laws, it was the sense of this Committee that the expression is figurative and no seal of approval is to be issued. The function of the Committee is chiefly that of therapeutic trial, and any approval is merely to be a recommendation based on clinical evidence.

On approval of the Executive Committee, the title of this Committee has been changed to "The Committee on Therapy" to conform to its wider scope.

A list of food adulterants to be obtained from the American Medical Association will be published by this Committee in THE JOURNAL OF ALLERGY.

Dr. Feinberg was authorized to address a meeting of pharmaceutical manufacturers and suggest that they meet with their committee to consider standardization of allergenic extracts, particularly pollen extracts.

The Committee approved the following by-laws and regulations: The Committee on Therapy shall consist of 12 members and fellows of the Academy of Allergy and such consulting members as may be nominated and elected by the members of the Committee. Each member shall be elected for a period of three years except during the year 1946-1947, during which time four members shall be chosen by ballot to serve one year and four to serve two years. Thereafter each year at the time of the annual meeting of the Academy the Committee shall nominate by two ballots, one individual to fill each vacancy created by the termination of membership. The first ballot shall require the presentation of two names by each member out of which the two individuals with the greatest number of votes shall be presented for the second ballot, from which the one with the greatest number of votes will be chosen as the new member. Members shall be eligible for renomination to the Committee.

The Committee shall meet each year at the time of the annual meeting of the Academy of Allergy and at such times during the year as may be required supplemental meetings may be called at the request of the majority of the members of the committee or upon direction of the executive committee or the Academy of Allergy.

There shall be elected each year by the Committee a chairman, vice-chairman, and secretary for the committee. Minutes of meetings shall be kept by the secretary and circulated to the members of the committee. Each officer may be elected to succeed himself, but for no longer than a period of three years.

The chairman should preside at each meeting; in his absence the vice-chairman. The secretary should notify the members of the committee in advance of the time of each meeting and of the subjects to be discussed.

The committee shall have as its major responsibility the investigation of drugs, foods, cosmetics, and devices used for the diagnosis, prevention, and/or treatment of allergy; the investigation to be initiated upon requests by members, or by others than members if the majority of the committee so approve by mail or vote; and of general measures, treatment of which may seem in need of study with a view to informing the profession. The results shall, if the majority of the committee so votes, be published in THE JOURNAL OF ALLERGY.

The constitution and by-laws of the committee may be amended at the time of the annual meeting if the majority of members so vote.

The committee voted and elected—

Chairman	Robert Chobot
Vice-Chairman	Harry Huber
Secretary	John Sheldon

SOUTHWEST ALLERGY FORUM

MARCH 31 AND APRIL 1, 1947

WASHINGTON-YOUREE HOTEL

SHREVEPORT, LA.

PROGRAM

Monday, March 31, 1947

9:00 A.M. "Some Faults in the Allergist's Routine," Dr. George L. Waldbott, Detroit, Mich. Discussion opened by Dr. H. Whitney Boggs, Shreveport, La.

9:30 A.M. "Pathology of Allergy—A Bridge to Understanding Clinical Allergy," Dr. Bernhard Steinberg, Toledo, Ohio. Discussion opened by Dr. L. O. Dutton, El Paso, Texas.

11:30 A.M. "Evaluation of Aerosol in the Treatment of Asthmatic Bronchitis," Dr. Boen Swinney, San Antonio, Texas. Discussion opened by Dr. Narcisse Thiberge, New Orleans, La.

12:30 P.M. Luncheon, Washington-Youree Hotel, Zephyr Room.

1:30 P.M. Round Table. "Nutritional Problems in Allergy." Leader: Dr. Robert Stone, Birmingham, Ala. Coordinator: Dr. Herbert Rinkel, Kansas City, Mo.

2:30 P.M. Greetings.

Clyde E. Fant, Mayor, City of Shreveport; Dr. C. Webb, representing the Shreveport Medical Society.

2:45 P.M. Round Table.

"Dermatological Allergy." Chairman: Dr. Dudley Youman, Shreveport, La.

1. "Where Is Allergy Indicated in Dermatology?" Dr. Dudley Youman, Shreveport, La.
2. "Non-Contact Causes of Allergic Dermatitis," Dr. Maurice C. Barnes, Waco, Texas.
3. "Contact Dermatitis."
   - A. "Plant Oils," Dr. Geo. T. Seibold, Houston, Texas.
   - B. "Cosmetics, Dyes, and Drugs," Dr. Ellis P. Cope, Little Rock, Ark.
   - C. "Preparation of Poison Ivy Extracts," Dr. Larry Halpin, Cedar Rapids, Iowa.

4:30 P.M. "Rationalization in Allergy," Dr. J. Harvey Black, Dallas, Texas.

6:00 P.M. Cocktail Party, Washington-Youree Hotel, Mezzanine Floor.

7:00 P.M. Banquet. Guest Speaker: Dr. Robert Stone, Nutrition Clinic, Hillman Hospital, Birmingham, Ala., "Vitamin Deficiencies—Emphasis on Recent Developments." Dr. Herbert J. Rinkel, Kansas City, Mo., "The Symphony of the Seasons." A Color Rhapsody of Winter, Spring, Summer, Autumn.

Tuesday, April 1, 1947

9:00 A.M. "Aerobiology: Evaluation of Air-Borne Allergens," Mr. O. C. Durham, Chicago, Ill. Discussion: Mr. H. L. Graham, Dallas, Texas.

9:45 A.M. Follow-Up Reports on Previous Papers, Dr. F. R. Rugeley, Wharton, Texas; Dr. Paul T. Petit, Beaumont, Texas.

10:00 A.M. Round Table.
"Treatment of Hay Fever." Chairman: Dr. Bernard G. Efron, New Orleans, La.
Leader: Dr. Mary Hewitt Loveless, New York City; Dr. French K. Hansel, St. Louis, Mo.; Dr. Oscar Swineford, Charlottesville, Va.
Coordinator: Dr. Francis M. Rackemann, Boston, Mass.
12:30 P.M. Luncheon. Washington-Youree Hotel, Zephyr Room.
1:30 P.M. Open Forum. "Evaluation of New Drugs in Allergy." Leader: Dr. Joe S. Shavin, Shreveport, La.
2:00 P.M. "Allergy in Relation to Cardiovascular Problems," Dr. Tinsley R. Harrison, Dallas, Texas. Discussion opened by Dr. Sim Hulsey, Fort Worth, Texas.
3:00 P.M. "The Classification of Asthma," Dr. Francis M. Rackemann, Boston, Mass. Discussion opened by Dr. Alan Cazort, Little Rock, Ark.
3:45 P.M. "Allergic Problems in Children," Dr. Jerome Glaser, Rochester, N. Y. Discussion opened by Dr. Ralph Bowen, Houston, Texas.
4:45 P.M. Business Session.

CENTRAL PENNSYLVANIA ALLERGY ASSOCIATION

The Central Pennsylvania Allergy Association will hold its Spring Forum on Allergy in Reading, Pennsylvania, on March 26, 1947. The speakers and their presentations are as follows:

Dr. Bela Schick—Lecturer on Disease of Children, Columbia University. "The Allergic Child and the General Practitioner."

Dr. Harold Abramson—Assistant Professor of Physiology, Columbia University. "Present Status of Allergy."

Dr. Harold Strause—Associate in Otolaryngology, St. Joseph's Hospital, Reading, Pennsylvania. "Ocular Allergy."

Dr. Carroll Wright—Professor of Dermatology, Temple University. "Treatment of Various Allergic Dermatoses."

Dr. Harrison F. Flippin—Associate Professor of Medicine, University of Pennsylvania. "What Price Sulfanilamide and Antibiotic Therapy?"

Dr. Ralph Mulligan—Chief of Departments of Allergy, St. Joseph's and Community General Hospitals, Reading, Pennsylvania. "Thirty Questions and the Allergist."

At the Morning Session at St. Joseph's Hospital, Dr. Stephen Locky will speak on the use of recent drugs in Allergy, and Dr. Arthur Kalish will discuss Dust Allergy. The Scientific Session will begin at 1:30 P.M. at the Berkshire Hotel and will be followed by a dinner.

NOTICE

The United States Veterans' Administration is announcing an approved residency of one year in Allergy at the Veterans' Administration Hospital, Aspinwall, Pa. This hospital is on a Dean's Committee, which provides regularly approved residencies in Internal Medicine.

Those interested in this position may apply to Dr. Lucien Gregg, Chairman of the Dean's Committee, School of Medicine, University of Pittsburgh.